
Bioremediation of Contaminated Soils - Aquifers on Reilly Site in St. Louis Park, Minnesota

Final Report
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by

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Table of Conents

Section 1. Sampling Protocols

Introduction.....	1
Overall Objectives.....	5
Sampling Protocols.....	7
Figures.....	13
Appendix.....	14
File Memorandum on Reilly Site Sampling, October 9, 1991	15
Analysis and Interpretation of Information from 10/9/91 Sampling.....	18
Map #1	19
Map #2	20
Log of Boring #SB-1	21
Log of Boring #SB-2	23

Section 2. Chemical Analysis

A. Analysis of Soil Samples.....	24
B. Soxhlet Extraction Procedures.....	25
C. Results of PAH Analysis	28
D. Discussion and Conclusions.....	29
Tables.....	32
Figures.....	35

Section 3. Biotreatability Study

A. Introduction	37
B. Biotreatability Studies Strategy.....	37
C. Stationary Bed Flow Through Bioreactor Studies.....	39
D. Saturated Column Studies of Microbial Transformation of PAH's in Mixed Soils	47
E. Degradation of PAH's Under Aerobic and Denitrifying Conditions	56
F. Column Study of 14C-Pyrene Biodegradation in Spiked Reilly Soil.....	60
Tables.....	71
Figures.....	74
Plates.....	90

Section 4. Split Spoon Insert Column Studies

A. Apparatus for Testing Minimally Disturbed Aquifer Materials	91
B. Column Preparation.....	92
C. Sampling Procedures.....	93
D. Effluent Analysis.....	94
E. Intermediate Column Samples	96
F. Column Soil Samples.....	97
G. Soxhlet Extraction and Analysis	98
H. SSIC Test Results	98
Tables.....	111
Figures.....	114
Plates.....	138

Section 5. Modeling Studies

Abbreviated Report on Modelling Transport in Porous Media with Biodegradation and Solubilization of Excess Substrate (TPMBX2).....	139
Abbreviated Report on Documentation, Application, and Evaluation of the TPMBX2 Program	140

Section 6. Recommendations

Recommendations	190
Figures.....	195

Section 1

Sampling Protocols

A. Introduction

1. BACKGROUND

A bioremediation treatability/feasibility study of the Reilly Superfund Site, St. Louis Park, MN has been carried out by the University of Minnesota under contract with the Minnesota Pollution Control Agency. This 80-acre tract was impacted by coal tar distillates (creosote) used for wood treating operations. Infiltration of chemicals have contaminated groundwaters and soils.

2. SITE DESCRIPTION

The Reilly Site is an 80-acre tract on which coal tar distillation and wood treatment operations were carried out for many years. The resulting contamination of soils, groundwater, and water wells have been documented elsewhere ¹. In early 1991, the status of the site was reviewed by staff from MPCA, MDNR, and U of M, with a view to assessing the feasibility of accelerating cleanup by insitu bioremediation. Major findings are summarized below.

Groundwater in the general area of the site has become contaminated as a result of releases of chemicals from a variety of activities.

- a. The swampy area south of the site is believed to have received discharges of wastewater and surface runoff contaminated with coal tar constituents that resulted in infiltration and groundwater mounding. This may have led to introduction of contaminated water into the drift aquifer, and also contaminated the peaty soil which underlies the swamp. Evidence of severely contaminated peat from this area was noted during early exploratory soil borings just north of Highway 7. Although the extent of this particular contamination problem was

¹Justin Blum's Report. Bioremediation of Creosote Contaminated Soil-Aquifer at St. Louis Park, Minnesota.

not defined, it is quite possible that it serves as a continuous source of groundwater pollution.

- b. Historical records suggest that drips, leaks, and spills on the site contaminated the surfacial soil on large areas of the site. The exact extent and distribution are not precisely defined. One of the earliest remedial actions on the site was aimed at moving some of the more severely contaminated soil and mounding it in an area on the southwestern side of the site. The mounded area therefore consists of a nonhomogeneous mixture of soils and debris that was skimmed from various parts of the site.
- c. A large plug of coal tar of unknown origin was found in the water supply well used by Reilly (W23 identified in reference #1 in footnote). Materials from this source are believed to be the cause of contamination of the Prairie du Chien-Jordan and the Ironton-Galesville aquifers. The plug of material has been removed but it is likely that there was concurrent contamination of adjacent soils which are now acting as sources of groundwater contamination.

The site and surrounding area is being pumped by source and gradient control wells. Pumped water is treated for discharge to municipal sewers. Pumping wells are located near the leading edge of the plume and are designed to control the spread of contaminated groundwater. The pumped water removes only small amount of source material and it is not known how long pumping will be required in the future.

Conclusions: there appears to be several source areas from which organic chemicals are leaching into the groundwater. The present pumping program is designed to control the spread of pollutants.

On-going pump and treat operations are not viewed as ecologically or economically sound long term solutions. All parties agreed that programs to eliminate polluting materials at the source to speed up the cleanup process and more importantly to minimize potential leakage of pollutants into interconnected aquifers are needed. In this connection, a study was proposed to examine whether source materials can be eliminated by onsite and/or insitu bioremediation.

Insitu bioremediation of source materials would have several advantages. It would mineralize organic pollutants, reduce/eliminate need for discharging groundwater to sewers, eliminate leakage of pollutants into interconnected aquifers, and eliminate a potential threat to regional groundwater systems.

3. BIOREMEDIATION THEORY

Bioremediation has been used effectively in a variety of situations ranging from surface sediments and near surface soils to deep aquifers materials. The underlying features are the same, namely.

- a. Soils and sediments usually contain a natural diversity of microorganisms capable of biodegrading a broad range of organic chemicals.
- b. Direct contact between pollutants and microbes is essential; it can be achieved by a combination of transport of cells and/or pollutants.
- c. Minimal concentrations of nutrients must be available to support biological oxidation.
- d. Aerobic biodegradation requires the presence of molecular oxygen or alternate sources such as hydrogen peroxide or nitrates.
- e. Anaerobic metabolism which does not require oxygen and produces methane as byproduct is possible but is not as versatile as aerobic biodegradation.

It is recognized that the physical state and distribution of the pollutants in the soil matrix is an important factor. Pollutants that are present as solutes in the groundwater are readily available. By contrast, chemicals that are present as a separate phase or sorbed on the surface of soil particles are not directly available for transport or biodegradation. Solubilization may be rate-limiting and must be taken into consideration in designing bioremediation strategies as discussed below.

- a. Sediments in ponds or basins that contain adsorbed or separate phase pollutants can be treated effectively in slurry type systems. Slurrying enhances contact between the pollutant and water which increases rates of solubilization. It is also

easier to control oxygen and nutrient addition in slurry systems.

- b. Contaminated surface soils can also be treated by slurring. However, slurring destroys the physical structure of the soil and makes ultimate disposal more problematical.
- c. Bioremediation in undisturbed soils (insitu treatment) relies on groundwater flow for contacting and for providing nutrients and/or oxygen. Injection of acclimated microorganisms with infiltrating water may be used to speed up the process.
- d. Bioremediation of pollutants that are held in the vadose zone may require control and manipulation of soil moisture content. Water is essential for solubilization and uptake of organic chemicals by microorganisms.
- e. When the chemicals are present as a separate phase, either solid or liquid, that fills pore spaces, rates of biological oxidation are determined by interfacial contact between phases. Solubilization occurs at the interfaces followed by biological oxidation. Colonization of the surfaces in the vicinity of the interface depends on the toxicity of the chemical and on availability of oxygen and nutrients. Information about the physical distribution of pollutant chemicals is therefore essential for formulating cleanup strategies.
- f. Oxygen requirements for aerobic biodegradation are directly proportional to the concentration of pollutants to be removed. Furthermore, oxygen is likely to be a limiting factor because groundwater oxygen concentrations are usually quite low. Oxygen can be supplied by recharging soil with reoxygenated water and/or addition of alternative sources of oxygen such as hydrogen peroxide or nitrates. Air ventilation of soils may be used to inject oxygen into soils. Soil air in the vadose zone is a potentially large supply of oxygen depending on rates of air exchange. Soil air venting can be used to replenish oxygen. Injection of air or oxygen below the water table is a potentially attractive mechanism for replenishing oxygen resources.

4. FOCUS OF TREATABILITY STUDIES

An important factor that constrained the scope of this investigation is that funding provided by MPCA was limited to a total of \$70,000.00. Furthermore, the project duration was limited to two years. Because of these constraints, it was necessary to focus and severely limit the scope of the research plan in terms of types and number of soil samples that were tested.

As noted in the Site Description section and the referenced reports, the Reilly Site contains a large variety of different soil type ranging from high organic content peat to low organic content clay, silt, sand, and gravel. The question of focusing the study therefore revolved on finding an appropriate location on the Reilly Site for taking representative samples for laboratory studies. Three factors were considered in making this choice, namely, (1) reviews of data from previous site investigations indicated that coal tar chemicals appeared to be the dominant pollutants in all contaminated soils, (2) that previous experience with biodegradation of creosote related chemicals showed that the intrinsic metabolic oxidation processes involved in biodegradation are not dependent of soil type, and (3), that rates of biodegradation are strongly influenced by the soil's physical properties because they dominate flow of water and air through the pores and therefore control the transport of nutrients, oxygen, and microbes.

Based on reviews of data from previous site studies and analysis of samples taken at four locations on the Reilly Site, a decision was made by MPCA staff to focus the study on soils of the mounded area of contaminated soils on the southwest side of the site. As indicated above, this mounded materials was known to consists of a nonhomogeneous mixture of severely contaminated soil taken from other parts of the site. It was mounded as a temporary storage area awaiting future destruction/disposal.

All subsequent soil samples were taken from the mounded site and analyzed to measure concentrations of coal tar related chemicals and to measure rates of solubilization and biodegradation in batch reactor tests and continuous flow column microcosms representing both minimally disturbed soils as well as columns loaded with well mixed soil samples.

B. Overall Objectives

A study of contaminated soils from the Reilly Superfund Site was undertaken to assess the feasibility of using insitu biodegradation technology to clean up the site and prevent further contamination of groundwaters. Insitu biodegradation is seen to be particularly appropriate for this site because coal tar chemicals are known to be biodegradable. However, it was deemed essential to carry out site specific laboratory studies because this site has impacted major aquifers that have been used as water supplies for a large populated area.

The studies were aimed at:

1. obtaining quantitative descriptions of the chemical composition and physical distribution of pollutants.
2. demonstrating biodegradability of the organic pollutants and developing quantitative descriptions of rates of biodegradation.
3. assessing the effects of limited availability of oxygen and nutrients and the potential advantages of supplementation of nutrients and oxygen.
4. correlating and interpreting the results in terms of engineering design concepts.

Specific objectives were designed:

1. To characterize the flow and transport of water, oxygen, and chemicals through representative, minimally disturbed, soil samples from various depth profiles in order to gain insight on insitu transport properties.
2. To obtain quantitative descriptions of the spatial distribution and chemical composition of chemicals. This data is needed to determine whether pollutants are present as a separate non aqueous phase liquid (NAPL), or as a separate non-homogeneously distributed solid phase, or as essentially uniformly dispersed adsorbed chemicals on the surface of sand grains. This information is essential for quantitative descriptions of rates of solubilization and their availability for biodegradation.

3. To measure biodegradation rates of specifically identified pollutants; this information is needed to answer the questions: (a) are the pollutants completely biodegraded and/or are intermediates released? (b) is biodegradation inherently limited due to lack of nutrients, oxygen, or capable organisms? (c) is biodegradation limited by soil matrix effects that control accessibility and transport of organic pollutants and/or microorganisms? (d) does biodegradation require the presence of cometabolites to initiate biodegradation?

Anticipating that more rapid cleanup of soils can be achieved by slurring the soil in water, MPCA staff also requested that data using this approach. However there was general agreement that the primary objective was to carry out column and microcosm tests to measure insitu treatment.

4. To assess the need for controlling pH, supplementing nutrients, oxygen and/or acclimated microbial cells to determine whether biodegradation rates can be increased significantly by supplementation.
5. The ultimate goal of this study was to develop quantitative descriptions of the interrelated effects of groundwater flow, solubilization, adsorption-desorption, transport, and biodegradation of organics that can be incorporated into models for use in engineering design studies of insitu bioremediation scenarios of the Reilly Site. The initial phase of the modeling study was based on using available mathematical models to correlate the column and microcosm data. However, as it became evident that the available models were inadequate for describing laboratory test results, new models had to be developed. Specifically, the new models were needed to incorporate the effects of changes in the spatial and temporal distributions of active cells, oxygen, and soluble chemicals on rates of removal. Furthermore, the new model had to incorporate the effects of solubilization of pollutants as a very critical step in cleanup of aquifers.

C. Sampling Protocols

This part of the report presents the results of the Reilly Site study concerned with characterizing the distribution of organic chemicals. It details the procedures used for obtaining soil samples and the analytical protocols employed for measuring the

composition and concentrations of organic pollutants associated with the soils.

1. SUBOBJECTIVES AND APPROACHES

The purpose of this part of the study was to develop quantitative descriptions of the composition and distribution of chemicals that are present. This information was needed for studies of insitu remediation strategies for removing creosote related chemicals. Specific compound identifications have focused on polynuclear aromatic hydrocarbons (PAH) because they are major constituents of creosote impacted subsurface environments, they are only slightly water soluble and therefore more difficult to remove, and some of the PAH's pose health risks. Non-specific measurements of organics were also carried out. They include measurements of total organic carbon by combustion and FID-GC measurements of the organics extracted by nonpolar solvents. Because creosote contamination is a very common problem, the study has stressed development of innovative protocols that may have applications at other sites.

2. SITE SAMPLING PROTOCOLS

The study has focused on the area referred to as the mounded area which was created as a temporary storage site for contaminated soils and was covered with top soil pending decisions regarding ultimate disposal. Because of the nonhomogeneous nature of the site a series of borings were carried out to obtain soil samples for testing.

Exploratory site sampling was carried out on October 9, 1991 with MPCA's drill rig. The purpose was to investigate the nature of the subsurface materials at various locations including the mounded area and the flat area between the mound and the pond. This type of information was necessary to establish procedures for obtaining representative materials and to develop protocols for taking minimally disturbed soil samples for laboratory testing. The result of this test work, including chemical analyses of the soil samples has been summarized in Section 1 Appendix (memo by L. Thai, 10/30/91. entitled Reilly Site Sampling).

Development of protocols for obtaining minimally disturbed samples of contaminated soils for testing in the laboratory turned out to be a critical initial

objective of this research program. Testing of minimally disturbed soils is deemed essential for defining rate data needed to assess the feasibility of insitu cleanup of contaminated aquifers. For this reason, a program for taking cores of aquifer materials by drilling with split spoon samplers with stainless steel inserts and adapting the filled tubes for column studies was undertaken. Needless to say, the procedures could have application at other sites. Construction and operating procedures of Split Spoon Insert Columns (SSIC) are described in a subsequent section.

3. REILLY SITE SAMPLING, DECEMBER 11, 1991

The purpose of the drilling excursion on December 11, 1991, was to obtain core samples and grab samples of soils from various locations on the mounded site at different depths. The core samples were designed for testing in the laboratory as column microcosms to obtain data on hydrogeological behavior of the soils as well as the chemical (e.g. types and quantities of contaminants, and their biodegradability) characteristics of the soil to help determine the feasibility of insitu biodegradation as a remedial action.

From the information obtained during the exploratory sampling in October, sites were chosen to be investigated further. The drilling was done by WTD Environmental Drilling of Minneapolis, Minnesota.

Locations of all the drill sites are shown on Map#1 in Section 1 Appendix, which also shows the locations at which soil cores were taken on December 11, 1991.

Figure 1.1 illustrates the sample gathering protocol for Drill Site #1 which is one of three borings carried out December 11, 1991. A seven inch flight augur was used to initiate drilling. Minimally disturbed soil cores were taken in the form of two feet long, 2.5 inch diameter, stainless steel, split spoon inserts at the depths indicated. In addition, grab samples of soil were taken at the locations indicated when the bore hole was widened between split spoon sampling takes.

Two Shelby tube samples were taken near the surface. But use of Shelby tubes was discontinued because the tubes were so severely damaged. Depth locations of the split spoon core samples and the grab samples are identified in Figure 1.1. It can be

seen that the split spoon sampling cores can be used to obtain an essentially complete vertical soil profile for laboratory testing. Test results on the grab samples and cores are presented in subsequent sections.

Additional information about the samplings at boring sites #1 and #2 are discussed below.

a. Boring #1:

The top 12 inches of soil was removed by shovel.

Shelby tube core samples were collected from the 1-foot to 3-foot, and 3-foot to 4-foot depths.

From 4-foot to 12-foot depth, split spoon core samples were collected every two feet.

From 14-foot to 20-foot depth, split spoon samples were collected every four feet.

The split spoon sampler yielded only water from 20- to 30-foot depth.

The last split spoon sample was obtained from 30- to 32-foot depth.

Grab samples were collected at 3,4,6,8,10,13,14,18,24. and 31.5 feet.

Shelby core samples were collected with 36-inch long Shelby tubes; and split spoon samples were collected with 3-inch diameter, 24-inch long split spoons with liners. Each split spoon core sample was capped at the ends by plastic covers and weighed. The weight was recorded on each tube along with the site name and depth. Grab samples were collected from the materials brought up by the frill after the drill was stopped at different depths. These samples were stored in 8-ounce glass containers with aluminum foil-lined screw caps which were labeled with the site name and depth. All samples (Shelby tube, split spoon, grab samples) were transported to the laboratory and stored in a constant temperature room set at 4°C.

b. Boring #2:

The second drill site was approximately 15 feet west of the first site. the drilling and sampling went as follows:

Split spoon samples were collected every four feet starting at the 1-foot depth (i.e. 1- to 3-foot depth, 5- to 7-foot depth, and so forth) and ending at the 25-foot depth.

Grab samples were collected at 5,9,13,15,23,25, and 30 feet.

The samples were treated as at the first drill site (i.e. labeling, weighing, and storing).

Water samples were also collected at these two drill sites. At both sites, the drainage was collected with a bailer after the drilling and soil sampling were completed. (The first bailer full of water was considered the drainage). At Site #1, the water samples were obtained from a depth of 25.6 feet. Besides the first bailer, the 5th and 24th bailers were also collected at Site #1. At Site #2, the first and tenth bailers, obtained from a depth of 30 feet, were collected as samples. The water samples were put into glass containers and these containers were stored in the 4°C constant temperature room with the soil samples.

After boring and sampling were completed, the excavated holes at both Site #1 and Site #2 were refilled with cuttings mixed with bentonite.

Borings were also made at Site #3 and #4. The samples from these sites were not used or analyzed. Several of the samples contained only gravel which prevented the filling of the cores. This was taken as an indication that the samples were not representative.

4. SPATIAL IDENTIFICATION OF SOIL SAMPLES TAKEN WITH SPLIT SPOON SAMPLER CORES AND CORRESPONDING GRAB SAMPLES

Dimensions of the split spoon samples are listed below along with a diagram of the dimensions of the flight auger that was used to widen the bore hole after each two foot split spoon sample was removed.

slit spoon sampler	3 in. I.D.
stainless steel insert	2 ⁵ / ₁₆ in. I.D.
flight auger	7 ⁷ / ₈ in. I.D.
auger tube	4 ³ / ₈ in. I.D.
auger wings	1 ³ / ₄ in. I.D.

As indicated above, the depth of each core was measured directly from the penetration of the frill stem. However, the depth location of the grab samples were adjusted to reflect the origin of the material that was brought up by the flight auger using the approach outlined below.

Let V_T = total volume of bore hole when auger was used to widen the 3-inch holes left by split spoon coring

$$V_T = \frac{\pi}{4} \frac{\left(7\frac{3}{8}\right)^2}{144} h_i \quad (\text{where } h_i = \text{distance cored in feet})$$

Actual soil volume removed by coring is less because $2\frac{5}{16}$ core was previously removed.

Let V_D represents the volume of soil that has been cored due to widening of the original 3-inch bore holes.

$$V_D = V_T - \frac{\pi}{4} \frac{\left(2\frac{5}{16}\right)^2}{144} h_i$$

Let V_S = volume of soil discharged at surface by the wings; it is assumed that some of the soil that is loosened by boring enters into the auger tube itself and is therefore not discharged at the surface, but is ultimately removed when the auger is removed from the hole. It is not clear how much soil was stored in the core as it penetrated the previous 3-inch hole. For this project (by hindsight) it was assumed that:

$$V_S = V_D - \frac{\pi \left(7\frac{7}{8}\right)^2 h_i}{4(144)} - \frac{\pi \left(4\frac{3}{8}\right)^2 h_i}{4(144)}$$

For future work, it is recommended that the soil volume that is captured in the flight auger core be measured in order to determine the amount of soil removed in order to more precisely determine the precise origin/location of the soil that is discharged at the surface. This may be important if the origin/location of the surface spoils that arrive at the surface are to be correlated with the split spoon sampling cores.

Section 1 Figures

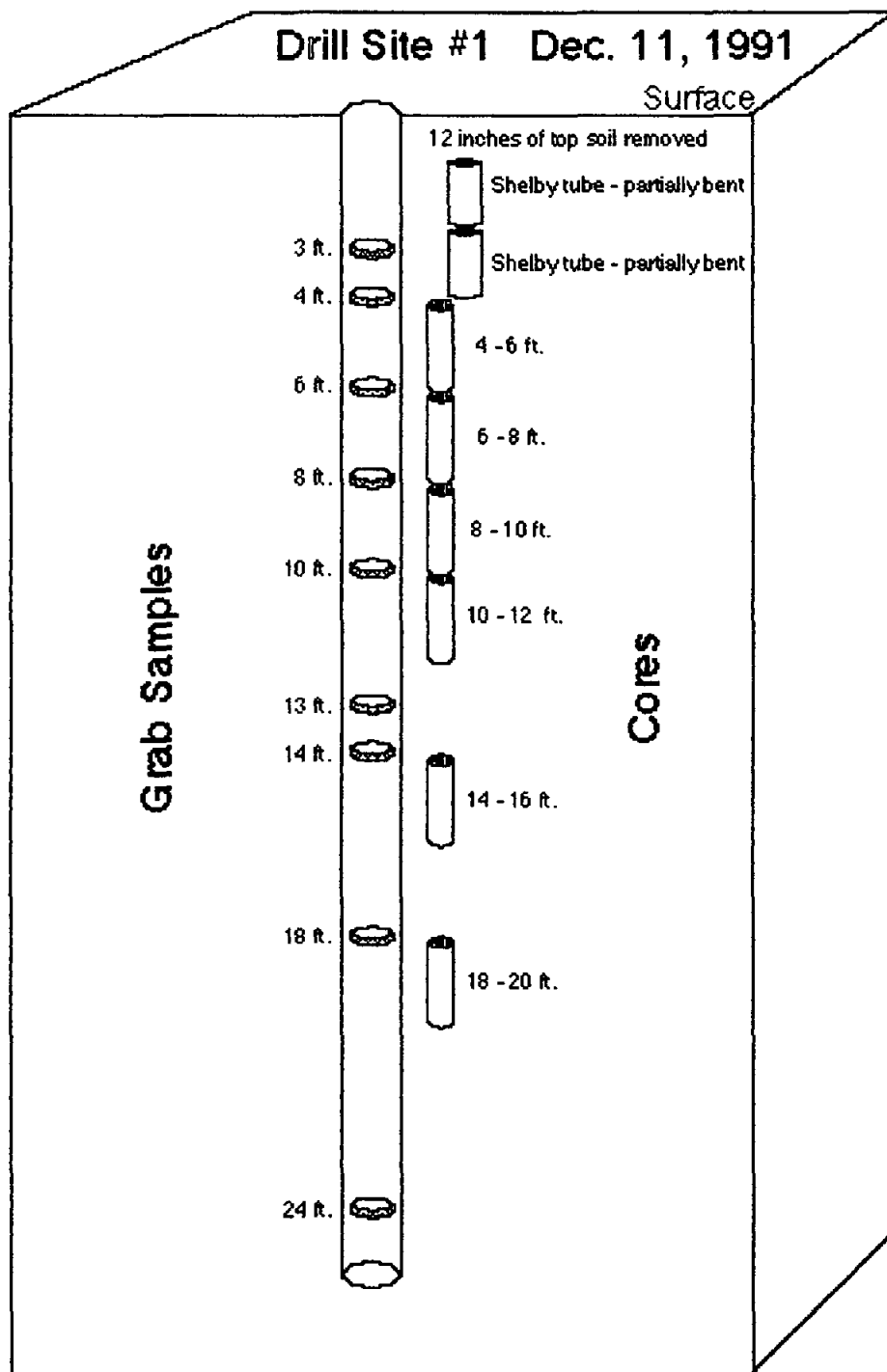


Figure 1.1 Sampling Protocol on 12/11/91 for Site #1 of the St. Louis Park Reilly Site

Section 1 Appendix

This appendix summarizes pertinent information about the Exploratory Sampling on October 9, 1991 and the planning for the subsequent sampling excursion on December 11, 1991.

It includes:

1. File memorandum dated October 30, 1991 that describes the locations of the borings. See MAP #1 as well as observations by the drillers.
2. A short analysis and interpretation of the data and observations of the sampling on October 9, 1991.

File Memorandum on Reilly Site Sampling, October 9, 1991 Using MPCA Drill Rig

Prepared by Le T. Thai

October 30, 1991

The purposes of the predrilling excursion on October 9, 1991, were: (1) to investigate the possible presence and extent of debris on Mound Reilly, as the debris, if it does exist, would have to be avoided during future drilling for core samples; and (2) to locate sites with high enough levels of contamination for worthwhile drilling of core samples.

Three sites were chosen during this predrilling trip to be investigated as possible drilling sites. The first was on Mound Reilly, the second on the flat area between Mound Reilly and the pond, and the third was south of Walker Street and north of Highway 7. The drilling was done by an MPCA drill rig with a hollow stem auger. Between drillings at different sites, the auger was cleaned with a solution of phosphate detergent and water.

The first drill site was on Mound Reilly (all drill sites are shown on the accompanying map.) The depth profile is as follows (see Map #1):

- The top foot beneath the grass was made up of fine tan clay.
- The next 7 to 8 feet (i.e. to depth of 8 to 9 feet below ground) consisted of black medium to coarse sand.
- From depth of around 9 to depth of 14 feet, the soil graded into a medium to fine-grained black clay (fill.)
- Starting at a depth of about 14 feet, there were coarse small stones mixed in with the black fill. This mixture extended down to a depth of 16 feet where we encountered about 5 feet of coarse to medium grained fill mixed with fine silty clay.
- Coarse alluvium of pea sized to 1-inch diameter stones in fine to medium black sand made up the rest of the drill depth. Drilling was stopped at the 29-foot depth where the ambient air concentration of volatile organics was 19 ppm.

After boring and sampling were completed, the hole (here as at the other two sites, also) was filled with cuttings mixed with bentonite.

At several depths, OVM readings were taken. OVM readings are measures of volatile organics concentration in the soil. At this site, volatile organics concentration increased to a peak of 84 ppm at a depth of around 8 feet, decreased to 55 ppm at 14 feet, and increased again to a maximum reading of 125 ppm around the 29-foot depth where drilling was stopped.

At all three sites, soil samples were taken at several depths. Duplicate samples were taken from the material brought up by the auger drills after stopping the auger at certain depths. These samples, which were stored in 8-ounce size glass containers with aluminum foil-lined screw caps, were transported to the laboratory in an ice chest and then stored in a constant temperature room set at 4°C. The samples were labeled according to their respective sites and depths. The soil samples will be analyzed for identification of contaminants and these analyses will help determine the depths at which core samples will be taken.

At Site #1 duplicate soil samples were taken at approximately every 5-foot interval during the drilling, starting at the 4-foot depth, and ending at the 29-foot depth. In addition, duplicate samples were taken at the 25-foot depth after the auger was pulled out after drilling was completed.

The second drill site is located on the area between Mound Reilly and the pond (see accompanying map.) The depth profile is as follows:

- Dark organic fill made up the first 2 feet (topsoil).
- Below the top 2 feet, the soil changed to a tan, medium to coarse-grained sand mixed with gravel down to a depth of 19 feet where drilling was stopped.

As at Site #1, OVM readings were taken at several depths. At this site, however, the readings were much lower than at Site #1, with a reading of 0.4 ppm in the top soil, increasing to a peak of 2 ppm at the 10-foot depth, and leveling out again to 1 ppm at the 19-foot depth.

Duplicate soil samples were taken at depths of 4, 9, and 14 feet. Also, samples were taken

at the 18-foot depth after the auger was pulled out of the ground after drilling.

Site #3 is located south of Walker Street, and north of Highway 7. This site, which was mainly peat, was highly contaminated. OVM readings were 90 ppm at the 6-foot depth, and 137 ppm at the 8-foot depth. Duplicate samples were taken at 2.5, 4, and 8 feet.

As mentioned above, all the soil samples will be analyzed (by Soxhlet extraction and gas chromatography) for identification of contaminants. From the drilling, it can be concluded there are sites for core samplings, namely, the site south of Walker Street and the site on Mound Reilly. The chemical analyses of the samples obtained at these sites will help determine at which depths core samples will be taken during the next drilling trip.

Analysis and Interpretation of Information from 10/9/91 Sampling

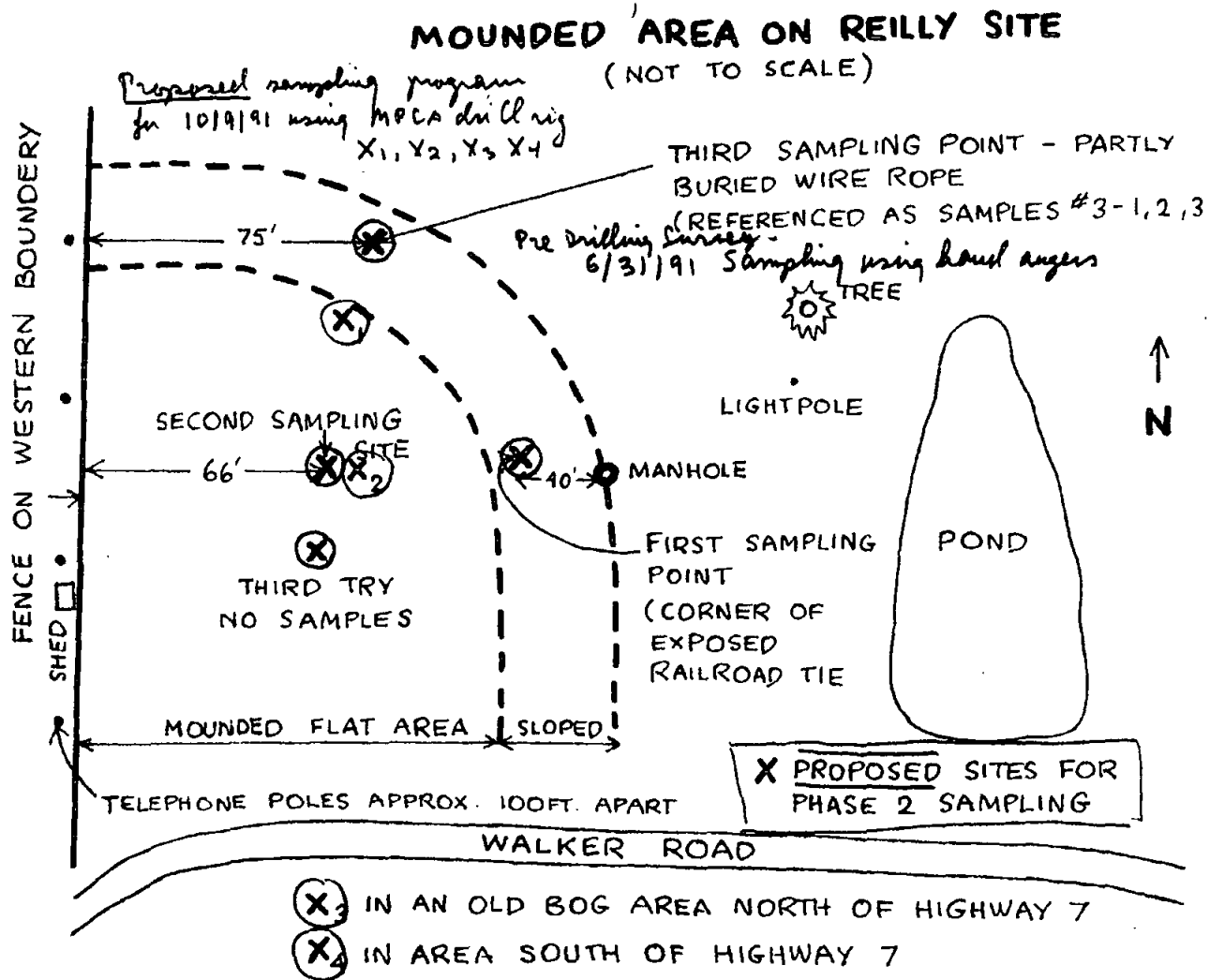
As discussed in the 10/30/91 memo ("File Memorandum on Reilly Site Sampling 10/9/91 by L. Thai) three locations were tested. The first drill site went to a depth of 29 feet. Measured PAH concentrations show a pattern of randomly distributed PAH concentrations. Highest concentrations occur in the 8-13 foot depth samples.

Relatively lower concentrations were measured at depths of 24 and 29 feet. This is consistent with subsequent drilling-sampling-analysis of the same general area of the site.

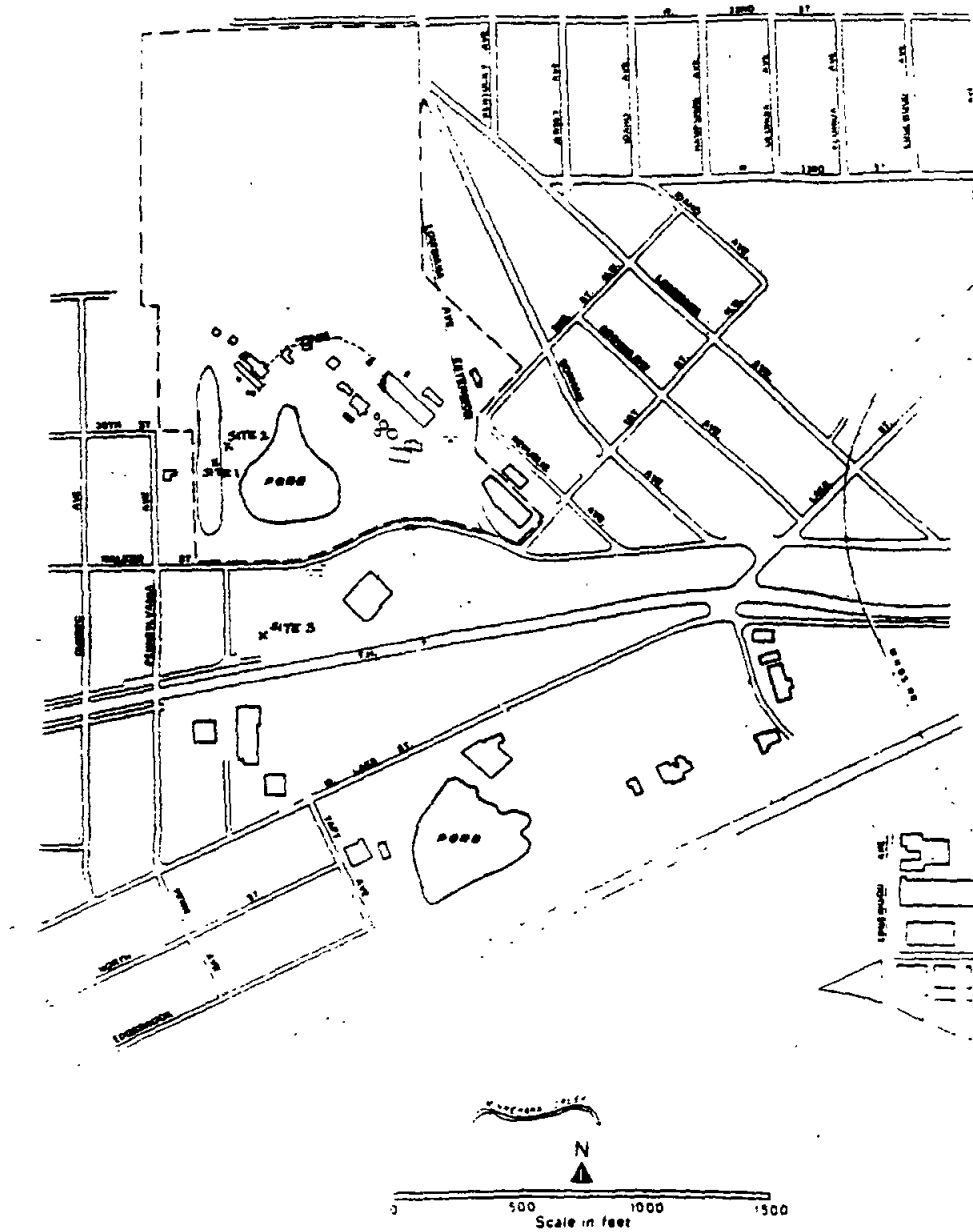
However, the observations recorded in the field show that there were strong odors and high concentrations of volatile organics even in the samples at 29 foot depths. OVM readings (organic vapor measurement) showed readings of 125 ppm on the spoils brought to the surface when the drill was approaching 29 feet. This is the highest value that was measured even though the spoils from the 8-13 foot depth contain significantly higher concentration of PAH's. This apparent mismatch between soil PAH measurements in the laboratory and OVM field measurements raises questions about the conclusions to be drawn.

In subsequent samplings, using split spoon cores, there was no evidence of high PAH concentrations or organic vapors (by smell) at depth approaching 29 feet. This apparent discrepancy is troublesome because it raises questions about how far down the contaminated soils extend. Do they extend into the water table? Or are the observation from the 10/9/91 sampling compromised due to intermixing of soils with more contaminated soils from above the nominal sampling point? The latter is beliwved to be a plausible explanation.

The question of whether there is significant soil contamination in the vicinity of the water table has important implications for cleanup of the site and the potential for offsite migration of PAH's into the east flowing groundwater.



Map #2 Area Map of the Reilly Site, St. Louis Park, Minnesota



Log of Boring #SB-1, Page 2, Minnesota Pollution Control Agency

Geology -

22	very coarse, pea size	↑	0.1m
23	to small stones. creaky texture, obvious,		79 ft. m.
24	gradually into a darker (dark) pebbles.	Coarse Alluvium	
25			
26	Layer of intermixing fine to medium sand & silt	↓	125 ft. m.
27	looks like		
28	red like top soil.		
29			
30			
31			
32			
33			
34			
35			

Log of Boring #SB-2, Minnesota Pollution Control Agency

MINNESOTA POLLUTION CONTROL AGENCY									
EPA ID NO.		PROJECT		LOG OF BORING NO.					
		Kally TAA		SB-2					
DEPTH FEET	DESCRIPTION (SOIL TYPE, GRAIN SIZE, COLOR, MOISTURE CONTENT, ETC.)			GEOLOGY	BLOW COUNT	SAMPLE TYPE	REC. INCHES	MINI OR OVA READING	OTHER
1	Dark organic fill (top soil)			fill					0.4 ft
2	Buff to tan, medium to coarse grained sandy gravel								
3									
4									
5	Buff to tan								1 ft
6	medium to coarse grained sandy gravel, light tan texture.								
7									
8									
9									
10	Sand			Nature Alluvium (coarse)					
11									
12									
13	Hit water								
14	Sand								
15	gravelly sand								
16	open to sand								
17	sandy gravel								
18	sandy								
19									
20									
21									

DEPTH	DRILLING METHOD	WATER LEVEL MEASUREMENTS							SURFACE ELEVATION
		DATE	TIME	SAMPLED DEPTH	CASING DEPTH	CAVE-IN DEPTH	DRILLING FLUID LEVEL	WATER LEVEL	
	Hand Operated								
	for Test					4.5			
BORING	DATE	TIME							
START	10/9/91	11:15							
COMPLETE	10/9/91	11:35							

LOG BY	RLH
OTHER	Hale
	filled with
	cuttings
	continues

Section 2

Chemical Analyses

A. Analysis of Soil Samples

PAH's listed below have been identified as the chemicals of concern based on previous surveys of the Reilly Site reported by MPCA, and the analytical protocols are designed to measure these PAH's:

Naphthalene	Phenanthrene	Benzo(a)anthracene	Benzo(a)pyrene
Acenaphthylene	Anthracene	Chrysene	Indeno(1,2,3-cd)pyrene
Acenaphthene	Fluoranthene	Benzo(b)fluoranthene	Dibenzo(a,h)anthracene
Fluorene	Pyrene	Benzo(k)fluoranthene	Benzo(ghi)perylene

Typical composition data for creosote (Table 2.1) shows that the predominant PAH's include some 17 compounds ranging from naphthalene with a solubility of 31.7 mg/L to Benzo(a)pyrene with a solubility of 0.003 mg/L. Phenolic and heterocyclic compounds are also present in creosote. However, the predominant phenols are very soluble as are most of the predominant heterocyclics. It is therefore likely that some, if not most, of these chemicals will have been solubilized and transported off site. Nevertheless, a study is underway to measure the total mass of organic chemicals that are present. More specifically, the total organic content as measured by combustion is being compared with the identified PAH concentrations to estimate unidentified mass of organic chemicals.

Several approaches have been pursued to quantitate the concentrations of non-PAH organics. Dissolved organic carbon in elution waters has been measured using a Dohrman TOC analyzer; these data give insight on concentrations of organics that are mobilized into the water phase hence become transportable.

Concentrations of nonpolar organics other than PAH's have also been determined from analysis of peak/areas of the GC-FID chromatograms that are used to measure PAH's. The peak/areas have been calibrated using dissolved carbon concentration measurements. Preliminary interpretation of these test data show that both types of measurements give consistent and directionally expected results based on mass balance calculations and measured oxygen consumption from biodegradation. The motivation

for pursuing these measurements is that the presence of additional organics affects the dissolved oxygen budget. It is therefore essential to incorporate this effect into the oxygen mass balance as described in the section in analysis and modeling.

The following 19 soil samples collected from the Reilly site were extracted and analyzed:

Sampling Date	Site#	Sampling Depths, ft
10/9/91	1	4,8,13,18,24,29
	2	2,9,14,18
12/11/91	1	3,4,6,8,10,13,14,18,24

B. Soxhlet Extraction Procedures

The Soxhlet Extraction procedures described under EPA method 3540 was followed. The method is for extraction of nonvolatile and semivolatile organic compounds from solids, sludges, and wastes, and is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures.

1. APPARATUS AND MATERIALS

- a. Soxhlet extractor:
40-mm I.D., with 500-ml round-bottom flask.
- b. Kuderna-Danish (K-D) apparatus:
Concentrator tube, 10-ml, graduated, Kontes.
Evaporation flask, 500-ml, Kontes.
Snyder column, 3-ball macro, Ace Glass, Inc.
- c. Boiling chips:
PTFE boiling stones, solvent extracted, Norton co.
- d. Extraction thimbles:
Cellulose extraction thimbles, single thickness, Whatman.

- e. Heating mantle:
3-sample model, individually rheostat controlled, Fisher Scientific Co.
- f. Reagent water:
Corning Mega-Pure water purification system.
- g. Sodium sulfate:
Granular anhydrous, purified by washing with methylene chloride followed by heating at 400°C for 4 hours.
- h. Methylene chloride:
OPTIMA grade, Fisher Scientific Co.
- i. 2-Fluorobiphenyl:
Surrogate, 1000 mg/ml in methanol.
- j. Glassware:
All laboratory glassware was cleaned according to the following procedures:
 - a. Laboratory grade detergent wash and rinse.
 - b. Multiple deionized water rinses.
 - c. Acetone rinse.
 - d. Oven dried (105°C) overnight.

2. SAMPLE PREPARATION

- a. Foreign objects such as sticks, leaves, and gravels were discarded from the soil sample.
- b. Ten (10) grams of soil sample were blended with 20 grams of anhydrous sodium sulfate until a dry homogeneous mixture was obtained.
- c. The mixture was transferred to an extraction thimble, and 100 μg of 2-Fluorobiphenyl was added to the soil by transferring 100 μL of the surrogate stock solution using a syringe.

- d. When moisture content of the soil sample was required, 10 grams of soil sample was weighed into a tared crucible and dried overnight at 105°C. The dried sample was weighed again after being cooled in a desiccator:

$$\% \text{ moisture} = \frac{\text{g of sample} - \text{g of dry sample}}{\text{g of sample}} \times 100$$

3. EXTRACTION

- a. Five hundred (500) ml of methylene chloride was added to the 500-ml round bottom flask containing one or two boiling chips.
- b. The flask was attached to the extractor and extracted for 16-24 hours.
- c. After the extraction was cooled and the extract cooled, the extract was concentrated to 10-ml using the K-D concentrator and the 3-ball Snyder column. The water temperature for concentration was adjusted (about 70°C) such that the concentration procedure was completed in approximately 30 minutes.
- d. The concentrated extract was then analyzed for PAH's in the gas chromatograph.

4. GAS CHROMATOGRAPH ANALYSIS

EPA method 8000 (General Gas Chromatography) and EPA method 8100 (GC Analysis of Polynuclear Aromatic Hydrogen) were followed.

- a. External Standard Calibration:

For each PAH of interest, calibration standards at a minimum of five concentration levels were prepared. One of the external standards was at a concentration near, but above, the method detection limit. The other concentrations corresponded to the expected range of concentrations found in real samples. Retention times were recorded for the identification of the analytes. Peak area responses were tabulated against the mass injected, and a

calibration curve for each analyte was prepared. A second-order curve fitting equation was obtained for each analyte.

b. GC Analysis:

Conditions for GC analysis were as follows:

GC:	Hewlett Packard 5890 Gas Chromatograph with auto-sampler
Integrator:	Hewlett Packard 3396A Integrator
Column:	Hewlett Packard HP-5, 25m x 0.2mm x 0.33 μ m
Detector:	FID
Carrier gas:	hydrogen, 2ml/min
Initial T°:	40°C
Initial time:	1 min
Rate:	5°C/min
Final T°:	300°C
Final time:	10 min
Injection vol:	2 μ L

C. Results of PAH Analysis

Results of PAH analysis of soil samples obtained from the St. Louis Park Reilly site on 10/9/91 and 12/11/91 are shown in Table 2.2. Sixteen PAH's have been identified in measurable quantities in most of the samples. Total concentrations are listed in the last column. All the profiles show vertical variations in concentrations. Analyses of replicates were carried out to determine the overall reproducibility of the Soxhlet-GC analysis procedure for measuring PAH concentrations of soils. The results as shown in Table 2.2 indicate that PAH measurements are very reproducible. Individual PAH's differed by less than 10% and total PAH concentrations differed by less than 2.2%. Duplicate tests were run on selected samples, so that measured differences in Table 2.2 are real and represent spatial variations in pollutant concentrations of the soils in the mounded site. The following discussion of these variations focuses on the Site #1 samples of 12/11/91.

Figure 2.1 present histograms of three concentration profiles. There are considerable variation in concentrations as a function of depth. The pattern is almost bimodal with

high levels at depths 4-6 feet and in the vicinity of 14 feet. It has been suggested that the variations are due to concentration differences of the original deposited materials that were obtained from different parts of the site.

It is interesting to note that the "% of Total" for each compound is relatively constant as a function of depth as shown in Table 2.3. This suggests that the chemical mixtures are from similar sources. Naphthalene shows the largest variations in the upper soil horizons. This may be the result of vapor losses and/or the more rapid rate of biodegradation of this compound. The bar graph in Figure 2.2 shows the same data to highlight the variations in naphthalene as opposed to the almost constant "% of Total" values for phenanthrene and Benzo(a)pyrene as a function of soil depth.

D. Discussion and Conclusions

1. The sampling and analytical protocols give quantitative descriptions of the concentration distributions of the 16 major PAH compounds. The random variations in concentrations with depth are consistent with the fact that the mounded area represents a layered mixture of contaminated soils from the whole site.
2. The similarity in the vertical percentage distribution of all PAH's indicates that the source materials from which these organics originated are quite similar. This information is useful because it gives support to the idea that the chemistry and kinetics of dissolution and biodegradation will be similar throughout the site.
3. The vertical variations in soil physical and chemical characteristics of the mounded storage area is not unexpected in view of the fact that the dumped soils came from different locations on the site. Inspection of the spoils brought up by auger drilling showed the presence of creosote soaked wood as well as mineral debris. Furthermore, random pockets of gravel and stone were detected during drilling operations indicating that this site is uniquely non-homogeneous both in terms of the size distribution of the inorganic soil constituents as well as the concentration of organic pollutants. Visual inspection of the spoils brought up by auger drilling also showed randomly distributed lenses of relatively clean sand without any detectable odor in contrast to black colored soil materials resembling silt-clay that had very

strong odor of creosote. Some peat like materials were also noted. In some instances, the drill had to be moved because of the presence of rocks.

The overall picture that emerges from these observations is that the mounded site is a random mixture of relatively clean and creosote contaminated materials. In this respect it is analogous to a landfill. However, it differs from a landfill in that creosote is essentially the only organic chemical of concern. Furthermore, although the measured PAH concentrations are significant, the total organic concentrations are relatively small being measured in mg/Kg of soil. Soxhlet extraction and GC analysis of small (10 g aliquots) of soil shows that the minimum and maximum concentration of total PAH is of the order of 9.3 to 1,650 mg/Kg soil. Treating these measured total concentrations as a random sampling of the site as a whole would indicate that the average total PAH concentrations are approximately 600 mg/Kg soil. At these concentration levels, oxygen and nutrients requirements for insitu biodegradation would be modest compared to the cleanup of landfills or contaminated sites that contain significant lenses of NAPL.

4. SOIL COMPOSITION NEAR WATER TABLE

The water table of the mounded area was not defined precisely. Based on the drillers' observations, saturated soils were encountered below 24 feet from the surface. It was noted that soil samples below 24 ft contained significant volumes of pea gravel. The samples had no detectable odor and no visible evidence of organic (black) contamination. Split spoon sampling was unsuccessful below the water table because the soil was not retained in the core except for one sample at 29 feet taken on 10/9/91 at drill site #1. As shown in Table 2.2 this 29 feet sample had relatively low concentrations of PAH's. It is noteworthy that the 24 feet sample had even lower concentrations. It appears from these results that soils below the water table are not contaminated significantly. Two explanations are offered, namely:

- a. The soils near the water table represent the original in place material, and this part of the site had not been impacted severely by creosote. But there may have been some downward mixing of deposited contaminated soils by the earth moving equipment.

- b. Lateral flow of groundwater is known to fluctuate seasonally and may have resulted in leaching of organic chemicals from the lower zone near the water table. This cleansing action has been operating over many years. It would be expected to be particularly effective in the vicinity of the water table horizon where alternative cycles of saturated and unsaturated conditions are conducive to aerobic degradation because of periodic contacts with soil air (oxygen). This scenario is consistent with the laboratory test results (discussed in the following sections) showing that there is complete aerobic biodegradation of PAH's when oxygen is not limiting.
5. It is recommended to carry out additional soil borings and testing of the site in order to describe more precisely the lateral extent of the polluted soil mass of the mounded area. Such information is needed for engineering planning studies aimed at describing the costs of cleanup of this part of the site. If such additional sampling and testing is to be carried out, it should be guided by a statistical analysis of sampling and analytical reproducibility in order to maximize the information that can be obtained from a minimum number of sampling.

Section 2 Tables

Table 2.1 Principle Components in Coal Tar Creosote

Predominant polycyclic aromatic hydrocarbons in coal tar creosote			
Compound	Relative percentage (wt)	MW	Aqueous solubility (mg/L, 25°C)
Naphthalene	13	128.2	31.7
2-Methylnaphthalene	13	142.2	25.4
Phenanthrene	13	178.2	1.3
Anthracene	13	178.2	0.07
1-Methylnaphthalene	8	142.2	28.5
Biphenyl	8	154.2	7.5
Fluorene	8	166.2	2.0
2,3-Dimethylnaphthalene	4	156.2	3.0
2,6-Dimethylnaphthalene	4	156.2	2.0
Acenaphthene	4	154.2	3.9
Fluoranthene	4	202.3	0.26
Chrysene	2	228.2	0.002
Pyrene	2	202.3	0.14
Anthraquinone	1	208.2	-
2-Methylantracene	1	192.3	0.04
2,3-Benzo(b)fluorene	1	216.3	0.002
Benzo(a)pyrene	1	252.3	0.003
Predominant phenolic compounds in coal tar creosote			
Compound	Relative percentage (wt)	MW	Aqueous solubility (mg/L, °C)
Phenol	20	94.1	82,000 (15°C)
O-Cresol	10	108.1	25,920 (25°C)
m-Cresol	10	108.1	23,500 (25°C)
p-Cresol	10	108.1	24,000 (40°C)
Pentachlorophenol	10	266.4	14 (20°C)
2,5-Xylenol	7.5	122.2	3,544 (25°C)
3,5-Xylenol	7.5	122.2	4,888 (25°C)
2,3-Xylenol	5	122.2	4,570 (25°C)
2,4-Xylenol	5	122.2	6,232 (25°C)
2,6-Xylenol	5	122.2	6,049 (25°C)
3,4-Xylenol	5	122.2	4,766 (25°C)
2,3,5-Trimethylphenol	5	136.3	-
Predominant heterocyclic compounds in coal tar creosote			
Compound	Relative percentage (wt)	MW	Aqueous solubility (mg/L, °C)
N-Heterocyclics and N-containing aromatics			
Quinoline	10	129.2	6,718 (20°C)
Isoquinoline	10	129.2	4,522 (20°C)
Carbazole	10	167.2	1 (20°C)
2,4-Dimethylpyridine	10	107.2	-
Acridine	5	179.2	5 (20°C)
Aniline	5	93.1	3,400 (25°C)
2-Methylquinoline	5	143.2	-
4-Methylquinoline	5	143.2	-
Pyrrole	5	67.1	-
Pyrrolidine	5	71.2	-
S-Heterocyclics			
Benzo(b)thiophene	10	134.2	130 (20°C)
Dibenzothiophene	10	184.3	2 (24°C)
O-Heterocyclics			
Dibenzofuran	10	168.2	10 (25°C)

Table 2.2 PAH Analysis of Soil Samples Obtained from the St. Louis Park Reilly Site on 10/9/91 and 12/11/91

Sampling Date	Site No.	Depth (ft)	PAH Concentration, mg per Kg Soil																	Total
			NAPH	ACNY	ACNE	FLUO	PHEN	ANTH	FLAN	PYRN	BAAN	CHRY	BBFN	BKFN	BAPY	INPY	DBAN	BPER		
10/9/91	1	4	40.12	7.17	22.23	32.57	75.54	59.81	51.06	37.89	19.24	22.78	15.94	8.95	16.37	24.38	19.86	19.60	473.51	
		8	92.04	6.16	75.77	69.74	163.97	54.09	126.16	89.23	29.65	33.97	19.93	15.49	18.23	20.78	16.76	17.03	849.00	
		13	42.21	10.32	31.58	33.18	91.08	31.88	107.88	86.66	33.41	45.52	30.87	21.94	28.36	29.05	20.30	25.39	669.63	
		18	36.88	6.64	33.15	33.34	78.04	33.11	75.50	58.37	24.21	30.01	20.15	17.89	19.42	23.94	19.69	20.06	530.40	
		24	6.33	1.29	8.64	8.80	25.25	9.89	22.23	16.72	5.94	7.88	5.05	2.80	4.59	4.58	3.09	4.16	137.24	
		29	10.09	1.42	11.00	10.95	31.21	12.19	26.29	19.52	6.54	8.50	5.51	2.86	5.00	4.88	3.12	4.22	163.30	
	2	2	1.47	1.38	0.87	1.89	4.78	3.70	6.34	5.80	3.65	5.59	5.24	2.57	4.05	6.23	3.34	7.04	63.94	
		9	0.24	0.13	0.28	0.48	1.59	1.29	1.45	1.55	0.58	1.06	0.62	0.52	0.43	0.57	0.35	0.51	11.65	
		14	0.35	0.22	0.79	0.75	2.59	2.65	4.88	4.00	1.57	2.43	1.59	1.21	1.29	1.12	0.51	1.01	26.96	
		18	0.24	0.15	0.29	0.27	0.61	0.48	1.29	1.09	0.54	0.98	0.71	0.39	0.52	0.65	0.39	0.67	9.27	
12/11/91	1	3	6.43	3.98	9.38	19.42	58.28	32.10	49.25	39.61	19.23	25.42	19.92	13.41	17.55	13.61	0.52	13.21	341.32	
		4	16.99	5.02	40.59	47.91	195.11	71.86	203.99	155.76	72.15	89.31	69.68	64.66	61.02	36.51	10.32	36.07	1176.95	
		6	80.63	2.99	75.15	74.83	195.49	116.51	131.24	94.97	38.96	51.49	34.82	ND	27.02	19.01	ND	18.80	961.91	
		8	61.14	2.64	62.92	54.84	135.16	60.49	133.82	99.83	44.59	63.19	45.26	21.69	34.46	22.19	ND	21.72	863.94	
		10	25.11	1.59	33.07	31.84	86.31	70.80	81.52	67.18	27.89	43.79	28.44	ND	21.83	13.42	1.45	15.10	549.34	
		13	62.51	10.10	49.17	47.83	148.94	60.89	161.77	136.26	69.34	112.49	74.96	64.08	63.78	37.69	13.61	36.77	1150.19	
		14	70.08	14.46	68.98	70.33	241.15	86.64	244.97	207.87	97.90	169.26	100.37	75.80	85.43	51.27	21.22	46.94	1652.67	
		18	58.08	6.61	50.19	46.02	153.06	72.60	158.47	132.49	59.80	88.91	57.52	51.91	48.94	30.48	9.20	28.58	1052.86	
		24	29.17	4.41	35.63	35.53	115.07	51.44	116.93	93.95	44.82	66.50	39.21	36.88	33.39	21.01	5.03	18.50	747.47	

Legends

NAPH Naphthylene
ACNY Acenathylene
ACNE Acenaphthene
FLUO Fluorene
PHEN Phenanthrene
ANTH Anthracene
FLAN Fluoranthene
PYRN Pyrene

BAAN Benzo(a)anthracene
CHRY Chrysene
BBFN Benzo(b)fluoranthene
BKFN Benzo(k)fluoranthene
BAPY Benzo(a)pyrene
INPY Indeno(1,2,3-cd)pyrene
DBAN Dibenzo(a,h)anthracene
BPER Benzo(g,h,i)perylene

ND Non-detectable

Table 2.3 Percentage Distribution of Total PAH's In Soil Samples from Site 1 of the St. Louis Park Reilly Site

Sampling Date	Site No.	Depth (ft)	% of Total PAH's															
			NAPH	ACNY	ACNE	FLUO	PHEN	ANTH	FLAN	PYRN	BAAN	CHRY	BBFN	BKFN	BAPY	INPY	DBAN	BPER
12/11/91	1	3	1.88	1.17	2.75	5.69	17.07	9.40	14.43	11.60	5.63	7.45	5.84	3.93	5.14	3.99	0.15	3.87
		4	1.44	0.43	3.45	4.07	16.58	6.11	17.33	13.23	6.13	7.59	5.92	5.49	5.18	3.10	0.88	3.06
		6	8.38	0.31	7.81	7.78	20.32	12.11	13.64	9.87	4.05	5.35	3.62	0.00	2.81	1.98	0.00	1.95
		8	7.08	0.31	7.28	6.35	15.64	7.00	15.49	11.56	5.16	7.31	5.24	2.51	3.99	2.57	0.00	2.51
		10	4.57	0.29	6.02	5.80	15.71	12.89	14.84	12.23	5.08	7.97	5.18	0.00	3.97	2.44	0.26	2.75
		13	5.43	0.88	4.27	4.16	12.95	5.29	14.06	11.85	6.03	9.78	6.52	5.57	5.55	3.28	1.18	3.20
		14	4.24	0.87	4.17	4.26	14.59	5.24	14.82	12.58	5.92	10.24	6.07	4.59	5.17	3.10	1.28	2.84
		18	5.52	0.63	4.77	4.37	14.54	6.90	15.05	12.58	5.68	8.44	5.46	4.93	4.65	2.89	0.87	2.71
		24	3.90	0.59	4.77	4.75	15.39	6.88	15.64	12.57	6.00	8.90	5.25	4.93	4.47	2.81	0.67	2.48

Legends

NAPH	Naphthylene	BAAN	Benzo(a)anthracene
ACNY	Acenathylene	CHRY	Chrysene
ACNE	Acenaphthene	BBFN	Benzo(b)fluoranthene
FLUO	Fluorene	BKFN	Benzo(k)fluoranthene
PHEN	Phenanthrene	BAPY	Benzo(a)pyrene
ANTH	Anthracene	INPY	Indeno(1,2,3-cd)pyrene
FLAN	Fluoranthene	DBAN	Dibenzo(a,h)anthracene
PYRN	Pyrene	BPER	Benzo(g,h,i)perylene

Section 2 Figures

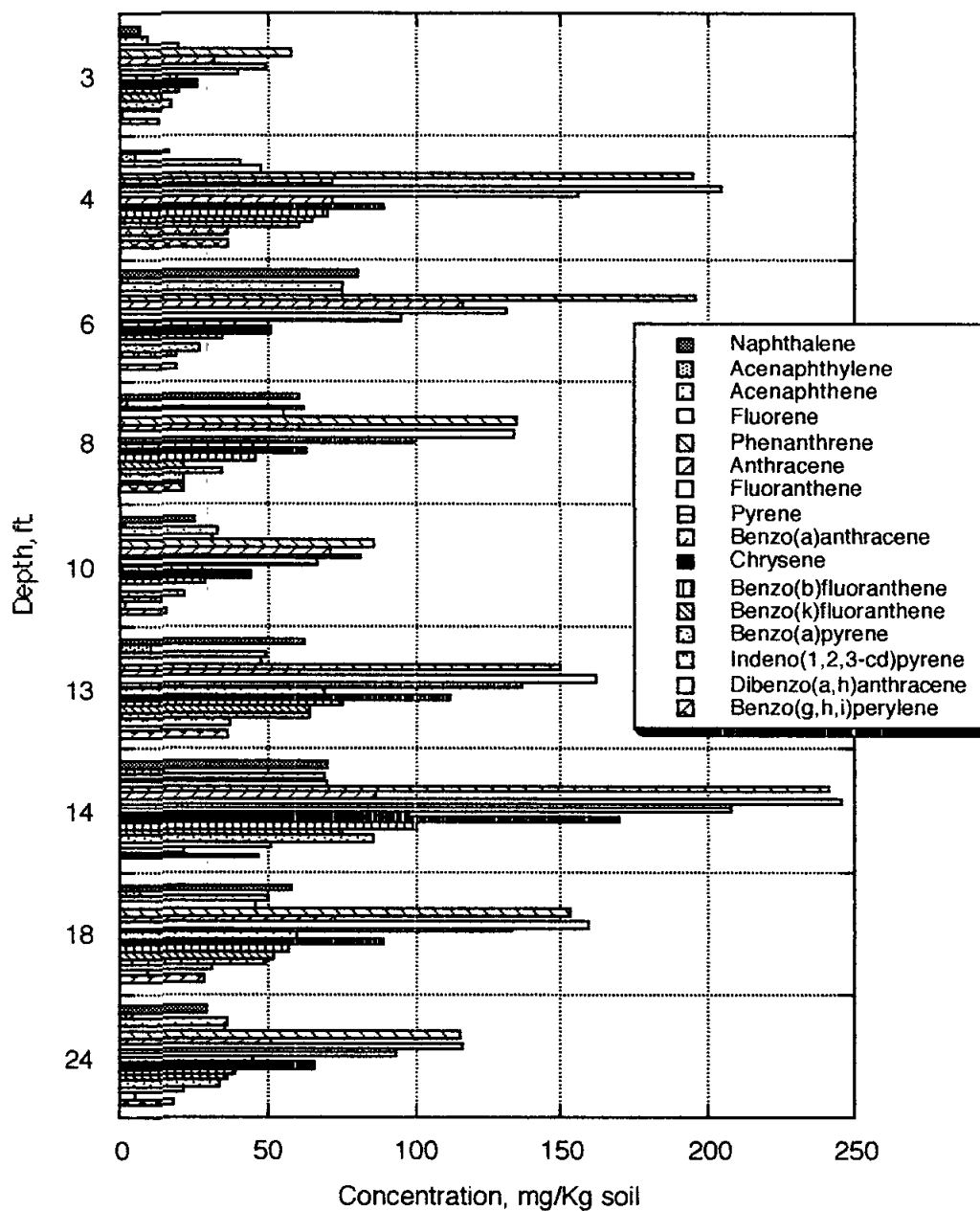


Figure 2.1 PAH Concentrations in Soil Samples from Site #1 of the St. Louis Park Reilly Site (Sample on 12/11/91)

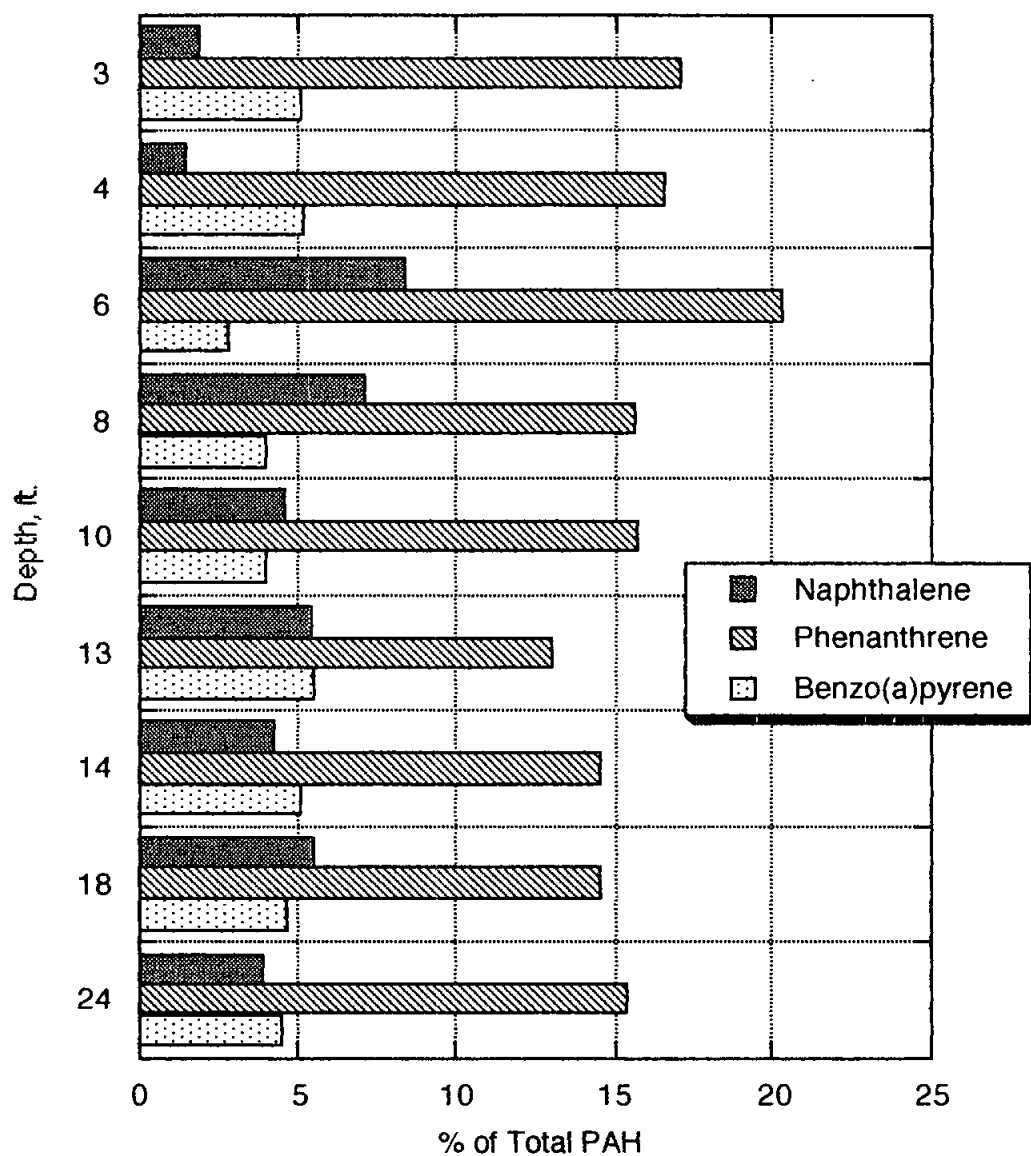


Figure 2.2 Distribution of Total PAH's for Naphthalene, Phenanthrene, and Benzo(a)pyrene in Soil Samples from Site #1 of the St. Louis Park Reilly Site (Sampled on 12/11/91)

Section 3

Biotreatability Study

A. Introduction

This part of the report describes the experimental results of the laboratory screening program for testing soils from the Reilly Tar Site in St. Louis Park, Minnesota. As indicated in Section 2 the soils contain primarily creosote type organic chemicals in which the polynuclear aromatic hydrocarbon compounds (PAH's) are the chemicals of concern. The screening program was designed to assess the potential of biological treatment for reducing the PAH content in soil.

B. Biotreatability Studies Strategy

1. BIODEGRADATION OF PAH'S IN SOIL

PAH's are neutral, non-polar organic compounds consisting of two or more fused benzene rings in linear, angular, or cluster arrangements. Due to the toxicity associated with the lower molecular-weight PAH's (White 1986, Pahlmann and Pelkonen, 1987), the U.S.E.P.A. has designated sixteen PAH's as being environmentally important and representative of PAH's as a class of compounds. These sixteen compounds are known as the EPA's Priority 16 PAH's. Table 1 includes some key characteristics of these compounds (U.S. EPA, 1980).

At present, many microorganisms are known to have the enzymatic capacity to catabolize PAH's that range in size from naphthalene to benzo(a)pyrene (Cerniglia 1984; Gibson and Subramanian 1984). Given the requisite environmental conditions, microbial communities are able to readily degrade these chemicals (Morgan and Watkinson, 1989; Meller et al 1989). However, experience gained from studies on biological soil remediation has shown that the degree of PAH degradation in different soils may vary significantly even under the same optimum growth conditions such as temperature, nutrients, oxygen supply and presence of sufficient population of PAH-degrading bacteria.

The soil physical and chemical characteristics play a major role in determining the rate at which PAH's are degraded. For example, the presence of elevated concentrations of heavy metals may result in the resistance of PAH's towards biological attack due to microbial toxicity. More significant is the effect of tight PAH binding to the soil organic phase and fine particles and clay content, resulting in a reduced substrate availability for microbial degradation.

The water solubility may also play an important role in their persistence in soil. As Table 3.1 indicates, the water solubility of the PAH's decreases significantly with the increase in molecular weight. This has a direct impact on their bioavailability to the microorganisms as carbon source. Thus, larger ring structure means less bioavailability, higher degree of recalcitrancy, and lower rate of degradation kinetics. Due to these forementioned variables affecting the site-specific performance potential of bioremediation, it is imperative that a laboratory biotreatability test be conducted to evaluate the feasibility of bioremediation for the Reilly Site.

2. LABORATORY STUDIES PROTOCOL

In U.S. E.P.A. Interim Guidance document titled "Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening, the sophistication and length of the laboratory program can be determined by three levels of objectives. The first and lowest level of testing is for "Remedy Screening". It is used to establish the validity of bioremediation to treat waste. The second and next level of testing is for "Remedy Selection", which is used to identify the actual performance potential of bioremediation and its ability to meet clean-up goals. The third and most intensive level of testing is for "Remedy Design", which entails optimization experiments and complete mass balance and biotransformation pathway evaluation.

For this project, the first level strategy of "Remedy Screening" was selected as the most appropriate program objective. The experimental protocols were chosen to measure rates of biodegradation under 3 different environmental conditions:

- a. using a well mixed stationary soil phase bioreactor to test soil samples under

conditions that provide good contacting between soil surface and the circulating water phase. This water soil environment is designed to facilitate solubilization and obtain PAH biodegradation results in an accelerated but non slurry manner (no movement of soil.)

- b. using a saturated column study with contaminated soil from the Reilly Site in order to simulate in situ soil environment below water table; this test condition is designed to obtain PAH leaching and biodegradation and oxygen demand information on soils undergoing continuous percolation in columns packed with a homogeneous mixture of soils that is representative of average soil conditions.
- c. using batch reactors to measure degradation of added PAH's in column effluent under aerobic and denitrifying conditions

C. Stationary Bed Flow Through Bioreactor Studies

1. OBJECTIVES

This phase of laboratory biodegradation study was conducted to evaluate the potential of bioremediation for soils containing polynuclear aromatic hydrocarbons (PAH's) from the Reilly Site in St. Louis Park, Minnesota. The experiments were designed and implemented in accordance with the "Remedy Screening" protocol specified in the U.S. EPA guidance document "Guide for Conducting Treatability Studies under CERCLA: Aerobic biodegradation Remedy Screening" (U.S. EPA, 1991). Specifically, the objectives of the treatability study is to formulate a biological treatment strategy based on the results of a biotreatability evaluation, and site characteristics. The overall goal of this project is to use bioremediation to significantly reduce the toxicity, mobility, and organic content of the contaminated soil at the Reilly Site.

2. MATERIALS AND METHODS

a. Soil Sample

Soil samples for the Biotreatability Studies were collected from depth 8' to 10'

and 14' to 16' from Drilling Site #1 which is one of three borings carried out December 11, 1991 (Status Report, 1992). Sample preparation involved manual screening with a number 10 (2.00-mm) sieve to remove stones and large debris and homogenized to ensure uniformity of soil. Duplicates of soxhlet extraction analysis were performed after mixing to determine the PAH's contamination level of the soil sample. PAH's compounds were analyzed by EPA Method 3540/8100 which are the standard Soxhlet extraction and GC analysis procedures for polynuclear aromatic hydrocarbons.

b. Inorganic Salt Media and Chemicals

The inorganic salts in the nutrients solution are 0.5 g $(\text{NH}_2)\text{SO}_4$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.17 g KH_2PO_4 , 0.65 g K_2HPO_4 , 0.03 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ per liter of sterilized double-distilled water. The pH of this solution was adjusted to 6.5 - 7.0 with either sodium hydroxide or hydrochloric acid. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, fluoranthene, and pyrene used in biodegradation and denitrification studies were obtained from Supelco Co. (Bellefonte, PA) and had chemical purities of at least 99%. All other chemicals and solvents used were analytical grade and purchased from Fisher Scientific Co. (Pittsburgh, PA.)

c. Bioreactor

Batch test reactors were made from pyrex glass with 1.5 in diameter and 8 in height (Figure 3.1). Each bioreactor consisted of two halves, and a stainless steel wire basket which could hold 20 grams of dry soil was suspended in the lower part of the bioreactor. The water phase was mixed continuously with a magnetic stirrer. The purpose of the suspended stainless steel wire blanket was to hold the soil stationary but immersed in the swirling water phase. This configuration was designed to minimize sand grinding by the stirring magnet but providing good contacting between the soil and water phase. The reactor halves were held together with a clamp. Three hundred milliliters of oxygenated inorganic salts media was added to each bioreactor before closing it with a cap which was connected to a manometer. Carbon dioxide was scrubbed out by lithium hydroxide in a suspended cup at the top. The bioreactors were stirred continuously by a magnetic stirrer. One poisoned control bioreactor was

prepared in the same manner as others except that 0.2 % (w/w) sodium azide and 0.05 % (w/w) mercuric chloride were also added to the solution as microbial inhibitor.

The rate of utilization of oxygen was measured manometrically. The bioreactors were periodically opened and refilled with pure oxygen on a weekly basis. Using pure oxygen rather than air minimized potential oxygen transfer limitations from the gas phase to the water phase. Twenty milliliters of aqueous samples were taken from bioreactors before aeration to determine the PAH concentrations and the volume of this sample was replaced with fresh inorganic salts media. PAH concentrations in both aqueous and soil phases were monitored by sacrificing an entire bioreactor through out the experiment period for required analysis. Soil sample in the suspended stainless steel blanket was air dried and duplicates of soxhlet extraction were performed to determine the residual PAH concentrations. Liquid-solid phase extraction, modified EPA Method 525, was used to determine the aqueous phase PAH concentrations.

d. Aqueous Phase PAH Concentrations Analysis

The presence of PAH components in the aqueous phase were determined by liquid-solid phase extraction, modified EPA Method 525. PAH components were extracted by passing aqueous sample through a Varian 500 mg Bond Elut Octadecyl (C18) cartridges (Varian, Sugarland, TX) and then eluted with 2 ml hexane solvent. Ten μ l of a surrogate spike, 2-Fluorobiphenyl 1000 mg/l in methanol, is injected into the aqueous sample. The spike is added to track the efficiency of the solid phase extraction of the samples. The solvent phase PAH concentrations were then analyzed by gas chromatograph analysis (see Analytical Methods).

e. Analytical Methods

For soil phase PAH concentrations, the Soxhlet extraction procedures described under EPA method 3540 was followed. The method is for extraction of nonvolatile and semivolatile organic compounds from solids, sludges, and wastes, and is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of

chromatographic procedures.

1. *Apparatus and Materials*

a. Soxhlet extractor

40-mm I.D., with 500-ml round-bottom flask.

b. Kuderna-Danish (K-D) apparatus

Concentrator tube, 10-ml, graduated, Kontes.

Evaporation flask, 500-ml, Kontes.

Snyder column, 3-ball macro, Ace Glass, Inc.

c. Boiling chips

PTFE boiling stones, solvent extracted, Norton co.

d. Extraction thimbles

Cellulose extraction thimbles, single thickness, Whatman.

e. Heating mantle

3-sample model, individually rheostat controlled, Fisher Scientific Co.

f. Reagent water

Corning Mega-Pure water purification system.

g. Sodium sulfate

Granular anhydrous, purified by washing with methylene chloride followed by heating at 400°C for 4 hours.

h. Methylene chloride

OPTIMA grade, Fisher Scientific Co.

i. 2-Fluorobiphenyl

Surrogate, 1000 mg/ml in methanol.

j. Glassware

All laboratory glassware was cleaned according to the following procedures:

- (i) Laboratory grade detergent wash and rinse.
- (ii) Multiple deionized water rinses.
- (iii) Acetone rinse.
- (iv) Oven dried (105°C) overnight.

2. *Sample Preparation*

- a. Ten (10) grams of soil sample were blended with 20 grams of anhydrous sodium sulfate until a dry homogeneous mixture was obtained.
- b. The mixture was transferred to an extraction thimble, and 10 μg of 2-Fluorobiphenyl was added to the soil by transferring 10 μL of the surrogate stock solution using a syringe.
- c. When moisture content of the soil sample was required, 10 grams of soil sample was weighed into a tared crucible and dried overnight at 105°C. The dried sample was weighed again after being cooled in a dessicator:

$$\% \text{ moisture} = \frac{\text{g of sample} - \text{g of dry sample}}{\text{g of sample}} \times 100$$

3. *Extraction*

- a. Three hundred (300) ml of methylene chloride was added to the 500-ml round bottom flask containing one or two boiling chips.
- b. The flask was attached to the extractor and extracted for 16-24 hours.
- c. After the extraction was cooled and the extract cooled, the extract was concentrated to 10-ml using the K-D concentrator and the 3-ball Snyder column. The water temperature for concentration was adjusted (about 70°C) such that the concentration procedure was completed in approximately 30 minutes.
- d. The concentrated extract was then analyzed for PAH's in the gas chromatograph.

4. *Gas Chromatograph Analysis*

EPA method 8000 (General Gas Chromatography) and EPA method 8100 (GC Analysis of Polynuclear Aromatic Hydrogen) were followed.

a. External Standard Calibration

For each PAH of interest, calibration standards at a minimum of five concentration levels were prepared. One of the external standards was at a concentration near, but above, the method detection limit. The other concentrations corresponded to the expected range of concentrations found in real samples. Retention times were recorded for the identification of the analytes. Peak area responses were tabulated against the mass injected, and a calibration curve for each analyte was prepared. A second-order curve fitting equation was obtained for each analyte.

b. GC Analysis

The solvent phase PAH's concentrations were then analyzed by a Hewlett-Packard Model 5890 gas chromatography equipped with an auto sampler, two injectors, and a flame ionization detector. Hydrogen was used as the carrier gas with a flow rate of 2 ml/min, and air (40 psi)

and hydrogen (16 psi) were supplied for the flame ionization detector. Nitrogen with a pressure of 25 psi was used as the make-up gas for the detector. PAH components in the solvent phase were separated on an HP-5 (Hewlett-Packard, San Fernando, CA) capillary column (length, 25 m; inner diameter, 0.2 mm; film thickness, 0.33- μ m). The temperature program was as follows: 40°C for 1 min. followed by a linear increase of 8°C/min. to 280°C where it was held for 10 min. Injector and detector were both maintained at 250°C. The concentrations of PAH components in the organic extract were calculated by comparing peak area obtained by duplicate, 2.0- μ L injections with those of standards for each compound.

3. RESULTS

Table 3.2 summarizes the overall results in terms of total PAH's. The killed control showed 7% removal whereas biotreatment for the 103 day period reduced PAH concentration from 742.6 to 300.3 mg/Kg. This is equivalent to a 60% reduction. Thus biodegradation was very effective. The slight reduction in the control can be ascribed to solubilization. Solubilization in this experiment was limited because the water was not exchanged.

Data in Table 3.3 show the extent of biodegradation of each of the PAH's in the treatment bioreactors as a function of time. First column in the table represents the initial soil phase concentration of 16 PAH's. The distribution by ring size in this sample showed approximately 1 % 2-ring, 18 % 3-ring, 48 % 4-ring, 27 % 5-ring, and 6 % 6-ring compounds. This ratio differs from the ratios of PAH's in fresh creosote which usually contain higher percentage of low molecular weight PAH's. Since compounds with two or three rings compounds are much more volatile and less recalcitrant than the high molecule weight compounds, this PAH profile suggests that the site contained substantially weathered PAH constituents.

After 25 days of incubation, one reactor was sacrificed and PAH concentrations of soil was measured. Although compounds with three or four benzene rings such as acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and benzo(a)anthracene exhibited over 57 % degradation, five or six rings compounds were biodegraded to a lesser extent in the same period of time. This can be

attributed to the availability of three or four ring compounds because those compounds have higher aqueous solubilities than the high molecule weight PAH's. For example, acenaphthene has an aqueous solubility at 25°C of 3.47 mg/L whereas benzo(a)pyrene and benzo(ghi)perylene have solubilities of 0.0038 and 0.00026 mg/L, respectively. In general, as the number of condensed rings increases, its aqueous solubility and microbial degradation extent decreases. The decrease of solubility directly affect bioavailability and biodegradation of these compounds.

4. DISCUSSION AND CONCLUSIONS

The observation that rates of removal are slower for the less soluble chemicals is consistent with theory, namely that chemicals must first be solubilized before they are transported into the microbial cells for metabolism.

With continued incubation after the first 25 days, the extent of biodegradation of every compound increased very slowly. The slower rates of removal with time (after the first 25 days) is probably the result of "aging" of the organic pollutants. Microscopic examination of the soils shows that most of the organic pollutants are present as separate phase materials. Some are also likely to be adsorbed on soil organics such as humic materials that were naturally present at the time when the soils were contaminated. It is theorized that the separate phase material dominates the initial rates of dissolution. As the residual composition of the separate phase material changes with time, the rates of dissolution slow down because the slightly soluble chemicals dominate the rate of solubilization.

For an aged contaminated soil sample, the amount of PAH's can be biodegraded is dependent on the extent of dissolution and desorption from the soil particle. Researches have suggested that PAH-binding within the soil matrix can greatly influence its bioavailability and biodegradability (Weissenfels et al, 1991). It has also been suggested that the organic matter content of soil is the single most important factor determining the sorption of hydrophobic molecules such as PAH's. As PAH's are characterized by extremely high organic partition coefficient (K_{ow}), which indicates poor water solubility values, organic matter is then an excellent sorbent for these compounds. At the beginning of incubation, the rate of dissolution and desorption was greater than the rate of biodegradation, thus the PAH's were bioavailable to the indigenous microorganisms. As incubation

continued, the available aqueous phase PAH's became less because of possible slow dissolution and desorption, and the biodegradation rate decreased.

The observation that rates of removal slow down as a function of time have important practical implications.

- a. It indicates that the time required to achieve a specific level of cleanup is strongly dependent of the residual soil concentration levels to be achieved.
- b. The higher molecular weight chemicals are removed more slowly than the more soluble chemicals.
- c. Except for a short initial period, rates of removal are likely to be dominated by rates of dissolution rather than rates of biodegradation.

These observations-conclusions obviously have important implications for the successful implementation of insitu bioremediation technologies on PAH contaminated soils. The level of cleanup of residual PAH concentrations that must be achieved will pretty much determine the time it takes to clean up the site. However, as will be shown in subsequent experimental results, once the rate of dissolution becomes the dominant process, insitu rates of biodegradation will be sufficient to prevent any offsite migration of the chemicals of concern. The time frame for cleanup therefore becomes unimportant because the pollutants on the site will have been contained and offsite migrations of organic chemicals into groundwaters will have been eliminated.

D. Saturated Column Studies of Microbial Transformation of PAH's in Mixed Soils

1. OBJECTIVES

Of the various processes that govern the subsurface movement of organic pollutants, microbially mediated reactions are possibly the most complex. The results shown in previous section revealed that, under aerobic and sufficient nutrient conditions, some of the lower molecular weight polynuclear aromatic

hydrocarbons (e.g., phenanthrene) were fairly rapidly removed, whereas high molecular weight compounds (e.g., Indeno{1,2,3-cd}pyrene) were eliminated at much slower rates. The observed eliminations were postulated to be mediated by the combined effects of solubilization and biological processes. In an attempt to bring laboratory experiments closer to conditions in natural groundwater systems, laboratory column studies were initiated to confirm this hypothesis. The objectives of this phase studies were to further investigate the microbial transformation of PAH's, the extent of PAH reduction in soil under saturated-flow conditions, and attendant oxygen requirement.

2. COLUMN STUDY PROCEDURE

Figure 3.2 shows the experimental setup for this study. Two stainless steel columns with a diameter of 5 cm and 15 cm in length were used. Each column has a screw cap with 1/8 inch connectors on both top and bottom and a tapered end surface inside of the column to facilitate smooth water flow. Fine mesh stainless steel screens were placed in the ends of the columns to prevent clogging with soil. These columns were packed with same contaminated soil described previously. Soil samples were wet packed into each column and were saturated with an upflow of distilled water. The columns were positioned vertically and connect to a peristaltic pump with stainless steel and Viton tubing. The velocity of liquid flowing through the packed columns was 0.651 cm/h, which approximates typical groundwater flow conditions.

The columns were dosed with an oxygen sparged inorganic salts solution. One column served as a control column which was dosed with the same water solution except that 0.2 % (w/w) sodium azide was added. The pH of leaching solution was adjusted to 6.5 - 7.0 with either sodium hydroxide or hydrochloric acid. The columns were operated at $20 \pm 2^\circ\text{C}$ to facilitate laboratory testing. This temperature is somewhat higher than in most subsurface environments but represents the water temperature in the near surface at the field site during the summer months.

Additional studies to examine the temperature effects were carried out in subsequent studies and are described in subsequent sections of the report. Column effluent were flowed through a stainless steel cell which hold an Orion Dissolved Oxygen Electrode for continuous oxygen utilization monitoring and the reading was indicated by an Orion SA 520 meter. A Teflon-coated magnetic stirrer was used in

this cell to facilitate complete mixing. Column effluent was then collected in a 300-ml amber glass bottle with a Teflon-lined septa cap. The bottle were connected to a solvent trap with a 22 gauge syringe and Teflon tubing to collect volatile compounds that may present in the effluent sample. The solvent trap were analyzed periodically and found no detectable PAH's.

3. RESULTS AND CONCLUSIONS (OXYGEN DEMAND)

Figure 3.3 shows the measured oxygen concentration in treatment column effluent. Due to the high oxygen demand observed during the first 56 days of operation, the effluent oxygen concentrations were measured close to anoxic level. Approximately 9 mg/L of oxygen was consumed in the treatment column during this period because air sparging gave a dissolved oxygen (DO) concentration of approximately 9 mg/L. After 56 days operation, air sparged buffer solution was replaced with oxygen saturated buffer solution resulting in inflow DO concentration in the range of 35+ mg/L. The effluent oxygen concentration stayed at anoxic level but gradually broke through after about 80 days operation. Air was then used from 100 to 108 and 139 to 165 days to investigate the effect of oxygen concentration in the influent. Results show that when air sparged (9 mg/L DO) solution was used effluent concentration decreased to near zero level, suggesting that an anoxic zone was created in the column. Once oxygen saturated solution was used again, effluent oxygen concentration increased indicating the whole treatment column was in an aerobic condition. Results also show the importance of oxygen supply to biodegradation process. (The effect of oxygen supply on effluent PAH concentration is discussed below.)

An oxygen mass balance was calculated from the measured influent and effluent DO concentrations. The cumulative total oxygen demand curve for treatment column is presented in Figure 3.4. The slope of the cumulative total oxygen demand curve represents the overall rate of oxygen consumption during the study period. Linear regression analysis of the cumulative total oxygen demand versus time is 6.08 mg oxygen per day and the r^2 is 0.98.

The theoretical oxygen demand for stoichiometric biological conversion of 16 PAH's to carbon dioxide and water are as follows:

Naphthalene	$C_{10}H_8 + 12 O_2 \text{ -----} > 10 CO_2 + 4 H_2O$
Acenaphthylene	$C_{12}H_8 + 14 O_2 \text{ -----} > 12 CO_2 + 4 H_2O$
Acenaphthene	$C_{12}H_{10} + 14.5 O_2 \text{ -----} > 12 CO_2 + 5 H_2O$
Fluorene	$C_{13}H_9 + 15.25 O_2 \text{ -----} > 13 CO_2 + 4.5 H_2O$
Phenanthrene	$C_{14}H_{10} + 16.5 O_2 \text{ -----} > 14 CO_2 + 5 H_2O$
Anthracene	$C_{14}H_{10} + 16.5 O_2 \text{ -----} > 14 CO_2 + 5 H_2O$
Fluoranthene	$C_{16}H_{10} + 18.5 O_2 \text{ -----} > 16 CO_2 + 5 H_2O$
Pyrene	$C_{16}H_{10} + 18.5 O_2 \text{ -----} > 16 CO_2 + 5 H_2O$
Benzo(a)anthracene	$C_{18}H_{12} + 21 O_2 \text{ -----} > 18 CO_2 + 6 H_2O$
Chrysene	$C_{18}H_{12} + 21 O_2 \text{ -----} > 18 CO_2 + 6 H_2O$
Benzo(b)fluoranthene	$C_{20}H_{12} + 23 O_2 \text{ -----} > 20 CO_2 + 6 H_2O$
Benzo(k)fluoranthene	$C_{20}H_{12} + 23 O_2 \text{ -----} > 20 CO_2 + 6 H_2O$
Benzo(a)pyrene	$C_{20}H_{12} + 23 O_2 \text{ -----} > 20 CO_2 + 6 H_2O$
Dibenzo(ah)anthracene	$C_{22}H_{14} + 25.5 O_2 \text{ -----} > 22 CO_2 + 7 H_2O$
Benzo(ghi)perylene	$C_{22}H_{12} + 25 O_2 \text{ -----} > 22 CO_2 + 6 H_2O$
Indeno(1,2,3-cd)pyrene	$C_{22}H_{14} + 25.5 O_2 \text{ -----} > 22 CO_2 + 7 H_2O$

The stoichiometric oxygen demand ranges from 12 moles of oxygen/mole of naphthalene to 25.5 moles of oxygen/mole of the high molecular weight PAH's. For an equal molar ratio the stoichiometric oxygen demand is 312.75 moles which is equivalent to 10,008 grams of oxygen for an equimolar mixture of the 16 PAH's which is in turn equivalent to 3,403 grams. The overall stoichiometric biological conversion ratio is then approximately 2.94 grams oxygen per gram of the equimolar mixture of PAH based on the equations shown above. Similar calculations have been made for the measured PAH concentrations on soils but will not be discussed here.

Analysis of the initial and final soil sample was performed to calculate an approximate mass balance of PAH's removed from the treatment column. Detailed description of this analysis will be discussed latter. The measured mass of oxygen consumed and the mass of PAH's removed in this study along with the calculated stoichiometric conversion ratio are shown in Table 3.4. Measured conversion ratio greater than the stoichiometric prediction was likely to occur due to the presence of other organic carbon source and possible abiotic oxygen demand. Since measured PAH concentrations in composite soil only represent about 4 % of total organic carbon as measured by combustion, oxygen demand exerted from the

biodegradation of other organic carbon should be included. It was not possible to determine whether there was any abiotic oxygen demand in this study.

The oxygen demand ratio of 7.3 is significantly larger than the stoichiometric value of 2.94 for an equimolar mixture of PAH's. Adjustments to reflect the actual molar concentrations of PAH removal have been shown to have very little effect on the stoichiometric ratio. It is therefore evident that oxygen demand is dominated by non-PAH related organics that are present in the soil. This is consistent with the above mentioned finding that measured PAH's in the composite soil account for less than 4 wt% of total organic carbon. The detailed chemical composition of the bulk of the organic carbon in this composite soil is not known. It appears likely that it includes a variety of phenolic and heterocyclic organic chemicals of the type known to be present in creosote. It probably also includes natural organic matter that is usually referred to as humic or peat like material. Although these materials are ultimately biodegradable, their rates of biodegradation are usually extremely slow and would not be expected to be significant factors in the oxygen demand observed in these studies.

Conclusions:

The engineering implications of these findings as regards the supply of oxygen needed for complete insitu biodegradation are:

- a. Oxygen demand will be significantly larger than that required for PAH removal alone.
- b. Site specific tests should be made to examine the oxygen demand ratios. Procedures for measuring the oxygen demand have been described.
- c. Although the data have not been included in this report, the analytical protocols used for measuring PAH's also give quantitative measurements of the concentrations of non-PAH organics. It is therefore possible to obtain more precise measurements of potential oxygen demand by interpreting the FID spectra of soil extracts to include the peaks of non-PAH chemicals. These FID measured peaks represent unidentified carbon sources where oxygen demand is apparently quite similar to that of PAH's. It is therefore recommended that total

FID carbon measurements be obtained from each PAH GC analysis as an indicator of total oxygen demand.

4. RESULTS AND CONCLUSIONS (PAH REMOVAL)

Figure 3.5 illustrates the effluent PAH concentration for both treatment and control columns. The ordinate on this graph is the sum of the PAH effluent concentration plotted versus time (days). Except for one short period of time, effluent PAH concentrations in the control reactor were higher than in the treatment reactor. However, similar downward trends in concentration were obtained from both the treatment and control column. After 25 days of operation, PAH concentration in the effluent from treatment column decreased at a much faster rate than that from control column, suggesting that PAH effluent concentration was becoming more affected by aerobic biodegradation condition. The concentration of PAH detected in treatment column effluent gradually decreased with time, so that after 80 days, the overall PAH level in the effluent was no longer detectable. This is recognized as the point in time when rates of solubilization have become rate limiting.

As is evident from Figure 3.5, the breakthrough of PAH concentration in treatment column effluent was detected when air sparged buffer solution was used from 100 to 108 and 139 to 165 days. The level of the breakthrough was close to the range before complete removal in the effluent. However, after oxygen saturated buffer solution was used again, the PAH concentration in the effluent decreased so that all PAH's were no longer detectable. After 170 days of operation, oxygen saturated buffer solution was used until the end of experiment and the breakthrough of PAH concentration was not observed.

PAH concentration in control column effluent was approximately 2 to 5 times higher than the concentration in treatment column. At the beginning of the experiment, PAH concentration was observed at about 0.3 mg/L and slowly decreased to a stable level of about 0.1 mg/L after 120 days of operation. This profile of concentration can be considered as if flushing is the only remediation action. Published results from field studies with pump-and-treat indicate similar trend that PAH concentration in groundwater could stay at a very stable level after a rapid decrease at the early stage of operation. This behavior is partly attributed to nonequilibrium desorption or slow dissolution processes related to slow diffusion

of the PAH's from solid to aqueous phase.

The measured effluent concentration history of 8 PAH's in the control and treatment columns are presented in Figure 3.6 to 3.13. Naphthalene and acenaphthylene concentrations in control column were very low throughout the experiment although both compounds have much higher aqueous solubility than other PAH's (Figures 3.6 and 3.7). This was attributed to the low soil phase concentrations of naphthalene and acenaphthylene, 5.8 and 5.2 mg/L, respectively. The concentration of these two compounds in treatment column effluent were rarely detected indicating that they were also readily biodegraded in aqueous phase. The other evidence that indicates naphthalene and acenaphthylene were completely biodegraded in aqueous phase was that there was no breakthrough of these two compounds when oxygen supply was changed from pure oxygen to air sparging of the feed water

Acenaphthene's concentration was the highest concentration of all the PAH's that could be measured in both control and treatment columns. Before acenaphthene was completely biodegraded in treatment column, both columns had similar effluent concentration. However, after influent changed from oxygen- to air-saturated solution at about 100 and 139 days, acenaphthene concentration in treatment column effluent increased to a level that was very similar to the range in control column. This concentration was then decreased immediately upon the addition of oxygen-saturated buffer solution, confirming that availability of oxygen resulted in PAH's biodegradation. The same phenomenon can also be seen in other PAH concentration profiles except that the extent of leaching during oxygen deficiency period was not dramatic like acenaphthene (Figures 3.8 to 3.13). The effluent concentration of phenanthrene, anthracene, fluoranthene and pyrene were similar in both columns before 40 days operation. However, in the treatment column these compounds were no longer detectable after 80 days. The decrease of PAH's with time probably indicates that the indigenous bacterial population in the treatment column and their activity increased over time.

The concentration of PAH's in soil phase were measured by Soxhlet extraction and gas chromatograph analysis of the initial and final soil samples in both treatment and control columns. Table 3.5 shows the measured soil phase concentrations.

As shown in Table 3.5, residual PAH concentrations after 203 days in treatment column were significantly lower than in the control column. Overall PAH reduction in the soil phase in both treatment and control columns were 64.6 % and 14.4 %, respectively. This indicates that bioremediation of contaminated soil removes 4.5 times more PAH's than flushing alone in the same period of treatment. Comparison with the residual PAH concentrations in the stationary bed flow through bioreactor study (see previous section), the extent of biodegradation for PAH's was very similar to the results from bioreactor study, although the length of experiment was twice as long. PAH's with 2, 3 and 4 rings had the highest percent removal and compounds with 5 and 6 rings showed less removal. As mentioned previously, this trend is most probably related to the differences in aqueous solubility of the PAH's. In general, as the molecular weight of a compound increases with additional rings, its aqueous solubility decreases accordingly. This decreased solubility inversely impacts bioavailability and biodegradability. Data from these studies also agree with results obtained by Bossert et al. and Mueller et al., which demonstrated differential biodegradation of similar chemicals during bioremediation of contaminated soils.

Conclusions:

The results of these studies demonstrate that in situ bioremediation can be studied in continuous flow column experiments or in stationary bed flow through bioreactors to simulate the transport and transformation of contaminated organic compounds under saturated groundwater flow conditions. The systems were used to study removal of polynuclear aromatic hydrocarbons (PAH's) under conditions typical for a groundwater saturated zone. The most important conclusions of this study are as follows:

- a. Under aerobic condition, all 16 PAH's investigated were microbially transformed in treatment column. The overall soil phase removal was 64.6 % over 203 days of operation.
- b. The extent of removal for 2, 3 and 4 rings compounds were much higher than 5 and 6 rings PAH's.
- c. PAH's are removed by solubilization into the percolating water flow. However, the water phase concentrations are much lower than the saturation concentration

of individual PAH's as shown in Table 3.6.

It is noteworthy that the lowest ratio values are observed with the more soluble chemicals whereas high ratios obtained with the least soluble chemicals. This phenomenon has been observed in several of our studies on the Reilly soils. It is therefore not an artifact or error in analysis.

One possible explanation is that dissolution of those chemicals that are present at low concentrations in the soil phase are hindered. thus soil phase concentration should be considered as a variable. However, differences in soil phase concentration alone can not account for the low ratios of some of the soluble chemicals.

Another possible explanation is that some chemicals are more strongly adsorbed (bound) to organic surfaces in the soil phase. this explanation is being evaluated in a related study (funded from other sources) of adsorption desorption of mixtures of PAH's and the results will be reported in the near future.

A third possibility is that a greater fraction of the lower molecular weight solubilized chemicals are being biodegraded by the soil bacteria. This explanation is being tested in related studies that will be reported on in the future.

In any event, interest in understanding the reasons for the low solubilities is that this could lead to finding methods for increasing solubilities that could be used to accelerate insitu treatments.

- d. The laboratory column studies allowed investigation of the temporal changes in effluent PAH's resulting from oxygen limitations. On the basis of the substantial decrease in PAH's observed during the experiment with high oxygen addition, microbial biomass and activity in the column is seen as a critical variable. Since PAH concentration in treatment column effluent was decreased to nondetectable level after oxygen supply increased, it is suggested that enhanced in situ bioremediation can eliminate contaminants off-site migration which is very useful for restoration of PAH's contaminated soil and groundwater.

E. Degradation of PAH's Under Aerobic and Denitrifying Conditions

1. OBJECTIVES

The broad objectives of this phase of the study were to measure the metabolic capacities of indigenous microbial populations from Reilly site soils for biodegradation of the major PAH's. A related objective was to examine the feasibility of using an alternative source of electron acceptor, namely nitrate, under denitrifying conditions. Because of the highly carbonaceous nature of creosote waste, the addition of an additional electron acceptor may be required to enhance biodegradation. The use of nitrate as an alternative source of electron acceptor could serve as a supplemented source of oxygen. Microbial degradation of PAH's using nitrates has been reported in the literature. However, the rate and extent of biodegradation may depend on the microorganism population as well as availability of oxygen. Since nitrate is highly soluble in water and high concentrations can be supplied with the injection water, it was tested as a possible source of oxygen (final electron acceptor) for the indigenous microbial population.

2. MATERIAL AND METHODS

a. PAH Stock Solution

PAH stock solutions were prepared by adding a known amount of seven pure PAH crystalline solids including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene to standard inorganic salts media and dissolving them using magnetic stirring in a closed glass container in the dark. The inorganic salts media was sterilized (104 kPa at 121°C for 30 min) prior to the addition of organic substrates. PAH's (Supelco Chemical Co., Bellefonte, PA) used were of the highest purity available (>99%). After being mixed for 12 hours at room temperature, these solutions were filtered through an extra-thick glass fiber filter (no. 66077 Gelman Science, Inc., Ann Arbor, Mich.) to remove undissolved PAH's.

b. Inocula Source

Effluent was collected from the previously described column study of Reilly Site soil and was used as inocula source. The culture was also tested to measure the number concentration of viable cells and to identify the major cell types. Numerous samples of effluent were streaked for microbial plate count using a conventional growth medium. The results indicated that $6.5\text{--}9.0 \times 10^5$ cells were present in 1 ml of column effluent. Samples of column effluent were also streaked for isolation on nutrient agar (Difco Laboratories, Detroit, Mich.). After 5 to 14 days of incubation at 28°C, colonies representative of each of the different morphological types were removed and single colonies were repeatedly purified on nutrient agar. Eventually, five morphologically distinct bacteria were isolated in pure culture and three were identified with OXI/FERM tube (Roche Diagnostic Systems, Nutley, New Jersey) as *pseudomonas maltophilia*, *pseudomonas cepacia*, and *achromobacter*.

A series of batch reactor tests were carried out to measure rates of biodegradation of the seven solubilized PAH's.

c. Aerobic Biodegradation Study

Column effluent from saturated column study was collected freshly and used as inoculum in this study. The PAH stock solution mentioned previously and column effluent were individually purged with oxygen for 30 min and then both solutions were combined at 1:1 ratio and loaded into two 3.8-L amber glass bottles equipped with a teflon-lined open-port cap. The presence of dissolved oxygen was verified by direct measurement with an Orion D.O. probe (Orion, Boston, MA). One bottle was poisoned with 500 mg per liter of mercuric chloride and 2,000 mg per liter of sodium azide as biocides to inhibit microbial growth. Samples were then stirred in the dark at room temperature.

Replicate samples from each bottle were withdrawn with a 30-ml gastight syringe from sampling port without opening the cap at designated time intervals. Measurements were performed to determine the dissolved oxygen concentration in the test samples. This confirmed the presence of sufficient oxygen for possible mineralization of the PAH compounds. The final dissolved oxygen

measurement showed that dissolved oxygen was present throughout the test. The presence of PAH components in the aqueous phase were determined by liquid-solid phase extraction, modified EPA Method 525. PAH components were extracted by passing 30 mL of aqueous sample through a Varian 500 mg Bond Elut Octadecyl (C18) cartridges (Varian, Sugarland, TX) and then eluted with 2 ml hexane solvent. The solvent phase PAH concentrations were then analyzed by gas chromatograph analysis (see Analytical Methods mentioned previously).

d. Denitrification Study

Column effluent and PAH stock solution were individually purged with helium for 30 min and then both solutions were combined at 1:1 ratio. Two media with different nitrate concentrations were prepared. The absence of dissolved oxygen was verified by direct measurement with a D.O. probe (Orion, Boston, MA). Samples were then prepared aseptically in an anaerobic glovebox to preclude intrusion of oxygen. All preparations were made when the glovebox was vacuumed and filled with 20 psi nitrogen gas. The combined solutions described above were transferred to the glovebox for sample preparation. Preliminary experiments have shown that this transfer will not contaminate solutions with oxygen.

Aliquots were transferred to 30-mL serum bottles and were crimp-sealed immediately without headspace by using Teflon-lined butyl rubber septums and open-port cap. Poisoned controls contained nitrate and 500 mg of mercuric chloride per liter and 2,000 mg of sodium azide per liter as biocides to inhibit microbial growth. Samples were shaken on an orbital shaker in the dark at room temperature. Throughout the experiment period, replicates from each set were sacrificed at designated time intervals. The presence of PAH components in the aqueous phase were determined by the same procedure described in aerobic biodegradation study. Test samples were analyzed for possible residual dissolved oxygen before sampling for the PAH compounds. These measurements confirmed the absence of oxygen in the denitrification samples.

3. RESULTS

Figures 3.14 to 3.20 show microbial degradation of PAH's under aerobic conditions (excess oxygen present). The aqueous-phase concentrations of all compounds decreased from initial values to non detectable levels in 80 hours. Controls containing 0.2 % sodium azide to inhibit biodegradation showed no significant loss in aqueous-phase PAH's concentrations through out the duration of the tests. The rapid initial rate of naphthalene removal probably reflects the combined effects of more rapid transport and biodegradation of this more soluble and lower molecular weight PAH's. However, it is noteworthy that all 7 PAH's were removed to nondetectable levels within a short time frame using the column effluent as inoculum without any other sources of organisms. These results clearly demonstrated that the site soils contain capable microorganisms, and PAH's are removed in relatively short incubation periods.

Figure 3.21 to 3.27 show results of microbial degradation tests with PAH's and nitrate as oxidizing agent under denitrifying conditions. Oxygen was removed from the reactor contents by purging the solution with helium for 30 min. Colorimetric measurements for dissolved oxygen were conducted while preparing samples in an anaerobic box. These measurements confirmed the absence of oxygen in the samples. Two nitrate concentrations, 82.35 mg/l and 41.18 mg/l $\text{NO}_3\text{-N}$, were tested. Naphthalene was the only compound degraded from an initial aqueous-phase concentration of approximately 7 mg/l to 1 mg/l and 6 mg/l under high and low $\text{NO}_3\text{-N}$ condition, respectively. An apparent acclimation period of 12 days was observed under high $\text{NO}_3\text{-N}$ condition. The aqueous-phase concentrations of other compounds did not change over the 60-day duration of the denitrification experiment. Controls remained at constant concentration for each condition tested. The observation that higher molecular weight PAH's are not biodegraded with nitrate as electron acceptor does not come as complete surprise.

Researches have shown that the presence of oxygen on the PAH's aromatic ring or ring substituent is apparently a basic requirement for cleavage of the aromatic ring under anoxic conditions (Atlas, 1981 and Bouwer, 1983). The most significant result of this study is the demonstration that biodegradation of naphthalene may occur under denitrification conditions, although this may require acclimation periods of 2 weeks. Furthermore, naphthalene may not be the only creosote related organic chemical that can be biodegraded using nitrate as an electron acceptor. However, the findings that 6 of the PAH's were not biodegraded in the presence of

nitrate indicates that reliance on nitrates as a supplemental electron acceptor is problematical. this suggests that in order to stimulate in situ bioremediation of PAH's contaminated soil, oxygen has to be provided as major electron acceptor.

F. Column Study of ^{14}C -Pyrene Biodegradation in Spiked Reilly Soil

1. OBJECTIVES

The previous bioremediation studies performed with materials from the Reilly Tar Site appear to indicate that bioremediation of the contaminants of concern is feasible. However, these studies have been more qualitative in nature and do not provide adequate data to design a full-scale bioremediation system, or data that will be able to assess the long-term effectiveness of an insitu bioremediation system. Hence several factors that may affect the effectiveness of bioremediation were evaluated in this phase of study.

Nutrient availability is frequently a limiting factor in biological treatment. As a result, nutrient amendments are frequently employed during bioremediation and biological treatability studies. Optimum nutrient concentrations are site-specific and must be determined experimentally. In this phase of study, nutrient amendments were performed to evaluate the impact of nutrient addition.

Groundwater and subsurface soil temperatures do not significantly change throughout the year and temperature control may be difficult for insitu bioremediation. It is important to evaluate the biodegradation and removal rates under the temperature condition representative of the Reilly Tar Site. A constant temperature incubator was used to conduct the experiment under temperature condition determined from field measurement.

Nutrient addition, temperature control, etc., are generally performed in order to encourage the growth of an indigenous microbial population capable of biologically degrading the contaminants of concern. If the naturally-occurring microbial activity is insufficient to achieve the required rates of biodegradation, inoculation is usually evaluated. Previous studies have provided some support for this approach by

demonstrating that under certain conditions, inoculation accelerated the rate and extent of organic contaminant biodegradation. In this phase of study, microorganisms enriched from site soil samples was used to evaluate whether the inoculation will enhance the rate and extent of biodegradation.

For column studies, radiolabeled PAH can be used to evaluate more precisely both biological and abiotic removal mechanisms. The production of $^{14}\text{CO}_2$ from contaminated soil spiked with ^{14}C labeled pyrene provides a very specific, sensitive procedure for monitoring the mineralization of the radioactive compound. With this technique it is possible to unequivocally establish whether a microorganism or a microbial population has the ability to mineralize a specific PAH. Experiment performance can be assessed using a mass balance approach capable of accounting for mineralization, transformation, volatilization, and residual concentrations. Through the addition of known amount of radiolabeled PAH, the fate of the contaminants as well as the rate of biodegradation and the extent of removal could be monitored. In this phase of study, radiolabeled pyrene was selected as the model compound due to its high affinity to the soil and moderately resistance to biodegradation.

The objectives for this phase of study are:

- a. To quantify the rate and extent of in situ bioremediation through direct measurement of radioactive carbon dioxide;
- b. To quantify the potential operating parameters of the insitu bioremediation system including the required input of oxygen and other nutrients and the inoculation of PAH-degrading microorganisms;
- c. To assess the impact of underground temperature measured at Reilly Tar Site on the rate and extent of insitu biodegradation; and
- d. To estimate the potential long-term cleanup goals for the Reilly soil and the time required to achieve those goals.

2. MATERIALS AND METHODS

a. Soil Samples

Soil samples for the Biotreatability Studies were collected from depth 11' to 14' from Drilling Site #1 which is one of three borings carried out December 11, 1991 (Status Report, 1992). Sample preparation involved manual screening with a no. 10 (2.00-mm) sieve to remove stones and large debris and spiked with 0.021 $\mu\text{Ci/g}$ radiolabeled pyrene at specific activity of 60 microcuries per millimole. This soil was then homogenized to ensure uniformity of soil. Duplicates of Soxhlet extraction analysis were performed after mixing to determine the PAH's contamination level of the soil sample. PAH's compounds were analyzed by EPA Method 3540/8100 which are the standard Soxhlet extraction and GC analysis procedures for polynuclear aromatic hydrocarbons (see Analytical Methods). The radioactivity associated with the organic extract from Soxhlet extraction was quantified by liquid scintillation spectrometry. Table 3.7 shows the initial concentrations of all PAH's in the soil matrix used in this phase of study. Soil samples were also analyzed by the Research Analytical Laboratory at University of Minnesota to characterize the chemical properties of the soil matrix that are relevant to determining the potential for bioremediation. Table 3.8 shows the results of this analysis.

b. Inorganic Salt Media and Chemicals

The inorganic salts in the nutrients solution are 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.17 g KH_2PO_4 , 0.65 g K_2HPO_4 per liter of sterilized double-distilled water. The pH of this solution was adjusted to 6.5 - 7.0 with either sodium hydroxide or hydrochloric acid. Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene used in the culture enrichment and chemical analysis were obtained from Supelco Co. (Bellefonte, PA) and had chemical purities of at least 99%. All other chemicals and solvents used were analytical grade and purchased from Fisher Scientific Co. (Pittsburgh, PA).

c. Isolation and enrichment of bacterial cultures

Soil samples contaminated with creosote chemicals was collected from the mounded area at the Reilly Tar Superfund Site. These materials were used as a source of inoculum to establish an enrichment culture in salt medium containing a mixture of 16 PAH's. Fifty milliliters of salt medium was transferred to a 200-ml screw-cap Erlenmeyer flask and inoculated with 1.0 g of creosote-contaminated soil passed through a no 10 sieve. Flasks were shaken at 150 rpm at room temperature. The resulting mixed culture grew to a high cell density within 5 days of incubation and was routinely transferred (5 % inoculum) to identical medium at weekly intervals. Following several such transfers, disappearance of undissolved PAH's was visually apparent.

When subcultures of this community were plated onto peptone medium, five morphologically distinct species were isolated as pure cultures. In addition, when subcultures were streaked onto carbon-free salt medium and sprayed with an ethereal solution of phenanthrene (0.2%), zones of clearing of the hydrocarbon substrate indicated their ability to utilize phenanthrene as a primary carbon source.

d. Microbial activity measurements

Twenty four clean, sterile 50-ml centrifuge tubes, fitted with Teflon-lined screw caps, containing 30 ml of salt medium with appropriate amount of dissolved naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene were inoculated with 1 ml of a cell suspension. Killed-cell controls were prepared by sterilizing cell suspension and adding 0.2 % (w/w) of sodium azide. Duplicate centrifuge tubes were removed at an appropriate interval and the contents were extracted by solid phase extraction (SPE) procedure for determination of PAH concentrations.

e. Column Study Procedure

Experiments were conducted with five stainless steel columns assigned from A to E (shown in Plates 3.1 and 3.2). Each column had a diameter of 5 cm and 15 cm in length and had a screw cap with 1/8 inch connectors on both top and bottom. A tapered end surface inside of the column is provided to facilitate smooth water flow. Fine mesh stainless steel screens were placed in the ends of the columns to prevent clogging with soil.

These columns were packed with same contaminated soil described previously. Soil samples were wet packed into column B, C, D, and E and were saturated with an upflow of distilled water. Soil in column A was wet packed with a salt medium containing 5 ml of enriched culture described previously. This inoculation process was used to ensure that enriched culture was distributed evenly throughout the column. The velocity of liquid flowing through the packed columns was 0.021 cm/h. Column A, C, and E were dosed with an oxygen sparged inorganic salts solution and column B was dosed with an oxygenated distill water. Column D served as a control column which was dosed with same inorganic salts solution except that 0.2 % (w/w) sodium azide was added. The pH of dosing solution was adjusted to 6.5 - 7.0 with either sodium hydroxide or hydrochloric acid. Column A, B, C, and D were operated at $20 \pm 2^\circ\text{C}$ and column E was operated at $9 \pm 1^\circ\text{C}$ which represents the groundwater temperature at the site in a constant temperature incubator. Table 3.9 shows the experimental conditions for each column.

The columns were positioned vertically and connect to a peristaltic pump with stainless steel and Viton tubing. Tubing from the outlet of each column was connected to both a stainless steel cell and a sampling port containing a luer-lock adapter through the use of a three-way ball valve. Effluent can be rerouted to the stainless steel cell when dissolved oxygen measurement is necessary. A Teflon-coated magnetic stirrer was used in this cell to facilitate complete mixing. Dissolved oxygen measurements were performed with an Orion Dissolved Oxygen Electrode and an Orion SA 520 meter. A 1-L amber glass bottle with open-top closure and Teflon septa was used to collect effluent samples from each column. Each sampling bottle was also connected to two 40-ml glass vials. Each vial has an inflow 22 gauge X 8.9 cm needle and an outflow 22 gauge X 1.27 cm needle and all vials were interconnected with 0.25 inch o.d. Teflon tubing. Twenty five-ml of 0.2 M NaOH was placed in each vial to trap possibly escaped radioactive carbon dioxide during sample collection. After sample collection, the aliquot was then acidified with 2 mL concentrated H_2SO_4 to lower the pH to about 1.09 and $^{14}\text{CO}_2$ released from the mineralization of pyrene can be trapped into those two vials through a vacuum source. After applied vacuum to the last vial, a 22 gauge X 7.5 cm needle was used to bubble air through the acidified aliquot in the sampling bottle and an outflow 22 gauge X 1.3 cm needle carried the CO_2 to the first vial through the Teflon tubing. Lab experiment had been conducted and determined that the optimal

trapping time is 8 minutes. 0.5 ml of the NaOH containing trapped $^{14}\text{CO}_2$ was then added to 10 ml Liquiscint solution (National Diagnostics, Somerville, N.J.) The scintillation vials were agitated and then left undisturbed for at least 24 h. The radioactivity of the samples was measured with an LS 1801 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, California.) Counts were converted to disintegrations per minute by use of a quench compensation curve. H-number versus percent efficiency were constructed by using ^{14}C -toluene internal standards, with acetone used as a quenching agent.

f. Aqueous Phase PAH Concentrations Analysis

The presence of PAH components in the aqueous phase were determined by liquid-solid phase extraction, modified EPA Method 525. PAH components were extracted by passing aqueous sample through a Varian 500 mg Bond Elut Octadecyl (C_{18}) cartridges (Varian, Sugarland, TX) and then eluted with 2 ml hexane solvent. Ten μl of a surrogate spike, 2-Fluorobiphenyl 1000 mg/l in methanol, was injected into the aqueous sample. The spike was added to track the efficiency of the solid phase extraction of the samples. The solvent phase PAH concentrations were then analyzed by gas chromatograph analysis (see Analytical Methods).

3. RESULTS AND DISCUSSION

In this study we chose to add ^{14}C -labeled pyrene to contaminated Reilly soil and measure mineralization. This is an extremely specific method that requires few manipulations at the end of the experiment, and yields nonsubjective results. The inclusion of the labeled PAH in contaminated soil tests the ability of the indigenous microorganisms to mineralize the specific substrate in the presence of the vast number of compounds in the contaminated soil. Pyrene was used to represent the polycyclic aromatic hydrocarbons for several reasons: it was known from other studies to be biodegradable; it was commercially available with ^{14}C in the C10 position (thus the molecule requires extensive biodegradation before $^{14}\text{CO}_2$ is released); it was less volatile and with a high affinity to the soil; and it is more representative of polycyclic aromatic hydrocarbons.

Figure 3.28 illustrates the breakthrough curves of ^{14}C radioactivity in the column effluents vs. pore volumes introduced into five test columns. We observed disparate

levels of breakthrough behavior in the different columns under different conditions. Column A, B, and C had very similar breakthrough rate for the first 80 pore volumes. After about 85 pore volumes, column A and C experienced a 15-pore-volume slowdown and then the rate of breakthrough increased again until 160 pore volumes of solution had been passed through both columns. The reason for this 15-pore-volume slowdown was unknown. After 160 pore volumes, daily total radioactivity in the effluents from column A and C were the same as control column D in which microbial activity was inhibited. At 430 pore volumes, nutrient solution used for column C was replaced with 20 mg/L of surfactant solution to increase the dissolution rate of spiked ^{14}C -pyrene. After the introduction of surfactant solution, total radioactivity from column C increased gradually till the end of the experiment. The breakthrough rate from column B increased rapidly from 0 to 120 pore volumes and then the rates decreased slightly, but it was still the highest rate among 5 columns, till the end of the experiment. Column D served as a control column in this experiment and the rate of breakthrough represented the condition that dissolution and desorption were the mechanisms to remove the spiked ^{14}C -pyrene from the soil. As is evident from Figure 3.28, the rate of dissolution in column D was constantly slow throughout the experiment. Breakthrough curve for column E showed a similar trend as column D before 200 pore volumes and then ^{14}C -radioactivity gradually increased with a rate similar to the rates from column A, B, and C before 80 pore volumes. After 320 pore volumes, the rate of ^{14}C evolved decreased slightly throughout the rest of experiment.

As shown in Figure 3.28, evolution of ^{14}C radioactivity occurred rapidly under all conditions that were studied here. Total recovery percentages of ^{14}C radioactivity were 41.7%, 84.6%, 39.7%, 8.0%, and 59.8% for column A, B, C, D, and E, respectively.

The effluent oxygen breakthrough curves generated under different conditions are shown in Figure 3.29. The influent oxygen concentration provided was around 36 mg/L and the concentration difference between influent and effluent indicated oxygen demand from abiotic and biological process in the column. The oxygen concentrations for column A and C were stayed less than 1 mg/L throughout the experiment, while column B and E exhibited a early breakthrough and maintained at either saturated or over-saturated level. Oxygen concentrations in column D effluents were checked periodically and showed very little consumption.

Figure 3.30 shows the mineralization time course of ^{14}C -labeled pyrene under test

conditions. ^{14}C -pyrene proceeded after a 25- to 45-pore volumes lag period for column A, B, and C as opposed to an extensive lag period, 200 pore volumes, exhibited by column E. It should be noted that although the initial rates of mineralization differed somewhat, very similar amount of ^{14}C -pyrene was mineralized from column A, C, and E and the greatest amount of mineralization occurred in column B. Table 4 shows the percentages of ^{14}C -labeled pyrene that was removed and mineralized in this experiment.

In contrast to $^{14}\text{CO}_2$ evolution curve from column B, which increased steadily throughout the experiment, the $^{14}\text{CO}_2$ evolution curves for column A and C exhibited plateaus after 170 pore volumes. This behavior is consistent with the impact of depletion of oxygen on biodegradation. The levels of oxygen concentration in the effluents from column A and C indicated that there was a small oxic zone in each column. Once oxic zone was created, aerobic degradation process started until the amount of bioavailable ^{14}C -pyrene was depleted in that section of column. The total oxygen demand depends not only on the biodegradation of PAH's but also on other type of organic matters. Since oxygen concentrations in column A and C never broke through, it was possible that oxic zone was created very slowly in each column. Once bioavailable ^{14}C -pyrene was consumed in oxic zone, the evolution of $^{14}\text{CO}_2$ would be very minimum if the formation of oxic zone is not fast enough. The effluent radioactivity was mainly due to the dissolution of ^{14}C -pyrene in the anoxic zone. Surfactant solution was used to increase the bioavailability of ^{14}C -pyrene in oxic zone and the evolution of $^{14}\text{CO}_2$ increased again.

The continuous evolution of $^{14}\text{CO}_2$ in column B was mainly due to dissolved oxygen breakthrough in the entire column at the early stage of experiment. This result is consistent with the finding from previous studies, which PAH's can be biodegraded more efficiently under aerobic condition. In contrast, the lag phase in column E stimulated a breakthrough of dissolved oxygen concentration because the biological oxygen demand was very minimum. Once the indigenous microbial population had acclimated to the low temperature, the oxic condition enhanced the biodegradation of bioavailable ^{14}C -pyrene. The amount of $^{14}\text{CO}_2$ evolved eventually plateaued at about 500 pore volumes.

As is evident from Figure 3.30, it should be noted that although the initial rates of mineralization of ^{14}C -pyrene differed somewhat, similar amount of $^{14}\text{CO}_2$ were

obtained from column A, C, and E. Between column A and C, inoculation of PAH-utilizing culture enriched from the Reilly Mount contaminated soil resulted in slightly more mineralization of ^{14}C -pyrene in column A, as determined by $^{14}\text{CO}_2$ production. This result suggest that indigenous microbial population in contaminated soil from Reilly Mound has acclimated to the contaminants of concern and is evenly distributed in the soil. It is evidence that the slow formation of oxic zone in column A and C hindered the extent of ^{14}C -pyrene biodegradation. In the oxic zone, readily bioavailable ^{14}C -pyrene from fast dissolution of spiked chemical were biodegraded quickly. The decrease of aqueous phase concentration stimulated more ^{14}C -pyrene to dissolve from soil surface and the mineralization proceeded continuously. Once dissolution or desorption process slowed down due to the effect of rate-limited desorption on transport, the evolution of $^{14}\text{CO}_2$ ceased. This behavior can be improved by using surface activating agent such as surfactant to make more ^{14}C -pyrene available for biodegradation. Since pyrene is only biodegradable under aerobic condition, ^{14}C -radioactivity measured in column A after slowdown of mineralization was ^{14}C -pyrene desorbed from anoxic zone.

The extensive lag period in column E suggested that indigenous microbial population required about 200 pore volumes of time to acclimate to the low temperature. This is a very important information for design or implementation of bioremediation system at the site in the future.

There was a disparate difference of the amount of $^{14}\text{CO}_2$ evolved from column B and A, C, and E. It is possible due to the amendment of nutrients in the influent for column A, C, and E. The addition of nutrients stimulated the activity of indigenous microbial population, resulting in a high demand of carbon source. This new demand decreased the extent of PAH biodegradation and stimulated the biodegradation of more of other organic matters. This is also the reason why the oxygen demand in column B was lower than column A, C, and E, regardless the dissolved oxygen breakthrough in column E. In order to test this hypothesis, the oxygenated water used for column B was replaced with the nutrient solution at 580 pore volumes. Figure 3.31 shows the change of effluent oxygen concentration from column B after the addition of nutrient solution. As is evident from Figure 3.31, nutrient amendment increased oxygen demand for about 35% and the trend was not stopped yet at the end of experiment. There was no significant change of $^{14}\text{CO}_2$ evolution within the period of test. Basically this test proved that the addition of nutrients increased the oxygen demand.

Tracer tests were performed to characterize fluid and contaminant transport in the test columns. The chloride tracer tests provided information on (1) fluid residence times, (2) the degree of breakthrough at outlet of columns, and (3) dispersion. Breakthrough curves for conservative tracer (chloride) under experimental conditions were shown in Figure 3.32, with the observed concentrations normalized to the injection concentration. Chloride was injected as a broad pulse having a duration of 24 hours. The tracer breakthrough in response to the broad-pulse chloride injection is apparent. The steady state fractional breakthrough reached 100 percent for all columns. The pore volumes required to achieve 50 percent breakthrough were 1.2, 1.1, 0.95, 0.98, and 0.98 for column A, B, C, D, and E, respectively. This result indicates that the packing of soil into columns were consistent and flow pattern in each column was very similar.

4. CONCLUSIONS

A laboratory column system was developed to simulate the insitu biodegradation of PAH under saturated groundwater flow conditions. The system was used to study the indigenous microbial mineralization of pyrene under different amendment conditions. The amendments tested in this experiment included: (1) inoculation of enriched culture from contaminated site, (2) nutrient addition, and (3) temperature effect. The most important conclusions of this study are as follows:

- a. Between column A and C, inoculation of PAH-utilizing culture enriched from the Reilly Mound contaminated soil resulted in slightly more mineralization of ^{14}C -pyrene in column A. This result suggests that indigenous microbial population in contaminated soil from Reilly Mound has acclimated to the contaminants of concern and is evenly distributed in the soil.
- b. The slow formation of oxic zone in column A and C hindered the extent of ^{14}C -pyrene biodegradation. In the oxic zone, readily bioavailable ^{14}C -pyrene from fast dissolution of spiked chemical were biodegraded quickly. The decrease of aqueous phase concentration stimulated more ^{14}C -pyrene to dissolve from soil surface and the mineralization proceeded continuously. Once dissolution or desorption process slowed down due to the effect of rate-limited desorption on transport, the evolution of $^{14}\text{CO}_2$ ceased. This behavior can be improved by using surface activation agent such as surfactant to make more ^{14}C -pyrene available for biodegradation.

- c. Due to the help of dissolved oxygen breakthrough, 84.6 % of spiked ^{14}C -pyrene were removed from the contaminated soil and 37.9 % were mineralized. This dissolved oxygen breakthrough stimulated more mineralization of ^{14}C -pyrene, resulting in more dissolution from the soil in the entire column.
- d. Low temperature condition extended the lag period 4 times longer, compared with 20°C columns, and yet the maximum amount of mineralization was about the same as column A and C.

Section 3 Tables

Table 3.1 Properties of Polynuclear Aromatic Hydrocarbons

Compound	Number of Rings	Molecular Weight	Aqueous Solubility (mg/L)	Log 10 Octanol-Water
Naphthalene	2	128	31.7	3.37
Acenaphthylene	3	152	3.93	4.07
Acenaphthene	3	154	3.47	4.33
Fluorene	3	166	1.98	4.18
Phenanthrene	3	178	1.29	4.46
Anthracene	3	178	0.073	4.45
Fluoranthene	4	202	0.26	5.33
Pyrene	4	202	0.135	5.32
Benzo(a)anthracene	4	228	0.014	5.61
Chrysene	4	228	0.002	5.61
Benzo(b)fluoranthene	5	252	0.0012	6.57
Benzo(k)fluoranthene	5	252	0.00055	6.84
Benzo(a)pyrene	5	252	0.0038	6.04
Dibenzo(ah)anthracene	5	278	0.0018	5.97
Benzo(ghi)perylene	6	276	0.062	7.66
Indeno(1,2,3-cd)pyrene	6	276	0.00026	7.23

Table 3.2 Overall Biotreatability Results from Stationary Bed Flow Through Bioreactor Study

	t = 0 days (mg/kg)	t = 103 days (mg/kg)
Control	742.6	690.7
Treatment	742.6	300.3

Table 3.3 Concentrations of 16 Polynuclear Aromatic Hydrocarbons during Stationary Bed Flow Through Bioreactor Study

compound	days of incubation					
	0 treatment	25 treatment	46 treatment	66 treatment	88 treatment	103 treatment
Naphthene	5.2	3.5	3.4	3.4	3.1	2.5
Acenaphthylene	4.5	4.3	4.1	3.7	3.2	2.7
Acenaphthene	31.0	3.2	3.2	3.3	2.6	2.5
Fluorene	20.3	3.3	3.0	3.8	2.5	2.3
Phenanthrene	39.5	11.2	10.3	13.7	8.5	8.1
Anthracene	42.5	14.3	9.1	16.2	6.6	6.0
Fluoranthene	120.0	27.0	24.8	23.4	18.8	22.0
Pyrene	98.6	42.9	33.4	27.8	19.4	34.2
Benzo(a)anthracene	58.1	23.9	18.4	16.3	15.2	18.8
Chrysene	82.5	45.1	42.6	33.2	29.1	42.8
Benzo(b)fluorene	59.9	43.1	43.2	36.3	36.2	33.3
Benzo(k)fluoranthene	52.7	32.2	28.5	23.1	23.0	35.4
Benzo(a)pyrene	50.0	36.3	39.5	33.6	37.3	36.9
Dibenzo(ah)anthracene	34.9	21.5	25.3	22.9	21.7	23.3
Benzo(ghi)perylene	13.4	7.8	8.6	7.4	7.9	7.4
Indeno(123-cd)pyrene	34.0	20.8	23.9	21.0	21.2	22.0

Table 3.4 Conversion Ratio of Oxygen Demand and PAHs Degraded

Mass oxygen consumed (mg)	1113.6 mg
Mass PAHs degraded (mg)	151.9 mg
Oxygen Demand Ratio (mg O ₂ / mg PAHs)	7.3 mg

Table 3.5 Concentrations of PAH's From Saturated Column Study

Compounds	t = 0 days (treatment and control)	203 days (treatment)	203 days (control)
Naphthalene	5.8	2.6	3.8
Acenaphthylene	5.2	4.2	4.0
Acenaphthene	31.5	2.9	23.3
Fluorene	21.9	2.7	16.8
Phenanthrene	46.1	8.4	33.6
Anthracene	46.5	6.2	35.3
Fluoranthene	131.3	24.2	108.1
Pyrene	107.0	20.9	88.6
Benzo(a)anthracene	57.9	16.0	53.2
Chrysene	81.8	31.8	71.9
Benzo(b)fluoranthene	50.8	31.3	50.7
Benzo(k)fluoranthene	41.3	22.8	40.0
Benzo(a)pyrene	39.8	29.2	37.8
Dibenzo(ah)anthracene	22.9	22.4	22.4
Benzo(ghi)perylene	8.2	7.6	7.8
Indeno(1,2,3-cd)pyrene	27.8	23.8	26.8

Table 3.6

Compound	Water Solubility (mg/L)	Highest Effluent Concentration measured (mg/L)	Initial soil concentration (mg/Kg)	Ratio of highest effluent concentration measured to water solubility of the pure compound
Naphthalene	31.7	0.006	5.8	0.0002
Acenaphthylene	3.93	0.0035	5.2	0.0009
Acenaphthene	3.47	0.25	31.5	0.072
Fluorene	1.98	0.09	21.9	0.045
Phenanthrene	1.29	0.103	46.1	0.08
Anthracene	0.073	0.065	46.5	0.89
Fluoranthene	0.26	0.035	131.3	0.13
Pyrene	0.135	0.033	107	0.24

Table 3.7 Concentrations Of PAH's from Saturated Column Study

Compounds	Concentration (mg/kg)
Naphthalene	5.8
Acenaphthylene	5.2
Acenaphthene	31.5
Fluorene	21.9
Phenanthrene	46.1
Anthracene	46.5
Fluoranthene	131.3
Pyrene	107.0
Benzo(a)anthracene	57.9
Chrysene	81.8
Benzo(b)fluoranthene	50.8
Benzo(k)fluoranthene	41.3
Benzo(a)pyrene	39.8
Dibenzo(ah)anthracene	22.9
Benzo(ghi)perylene	8.2
Indeno(1,2,3-cd)pyrene	27.8

Table 3.8 Characterization of Soil for ¹⁴C-Pyrene Column Study

Total P (mg/Kg)	Total N (%)	pH	Organic Matter (%)	Total Carbon (%)	foc (%)
312.1	0.08	6.75	4.4	2.67	1.9

Table 3.9 Experimental Conditions of ¹⁴C-Pyrene Column Study

Column	Condition
A	Culture inoculation, oxygenation, and nutrient amendment
B	Oxygenation
C	Oxygenation and nutrient amendment
D	Oxygenation and microbial activity inhibition
E	Oxygenation, nutrient amendment, and low temperature

Table 3.10 Percentage of Removal and Mineralization of ¹⁴C-Labeled Pyrene

Column	A	B	C	D	E
%removed	41.7	84.6	39.7	8.0	59.8
% mineralized	13.0	37.9	13.6	--	14.5

Section 3 Figures

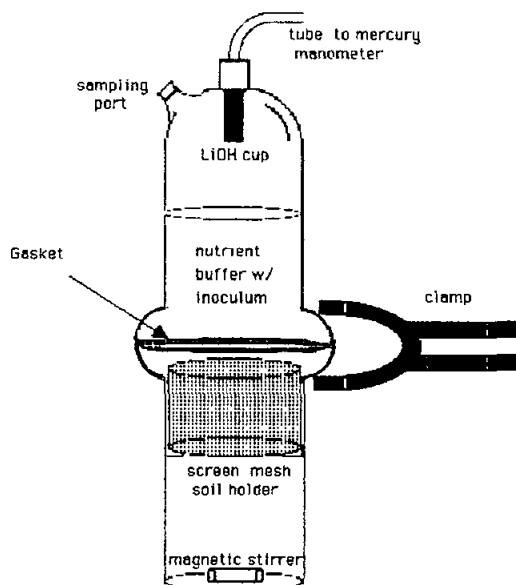


Figure 3.1 Stationary Bed Flow Through Bioreactor

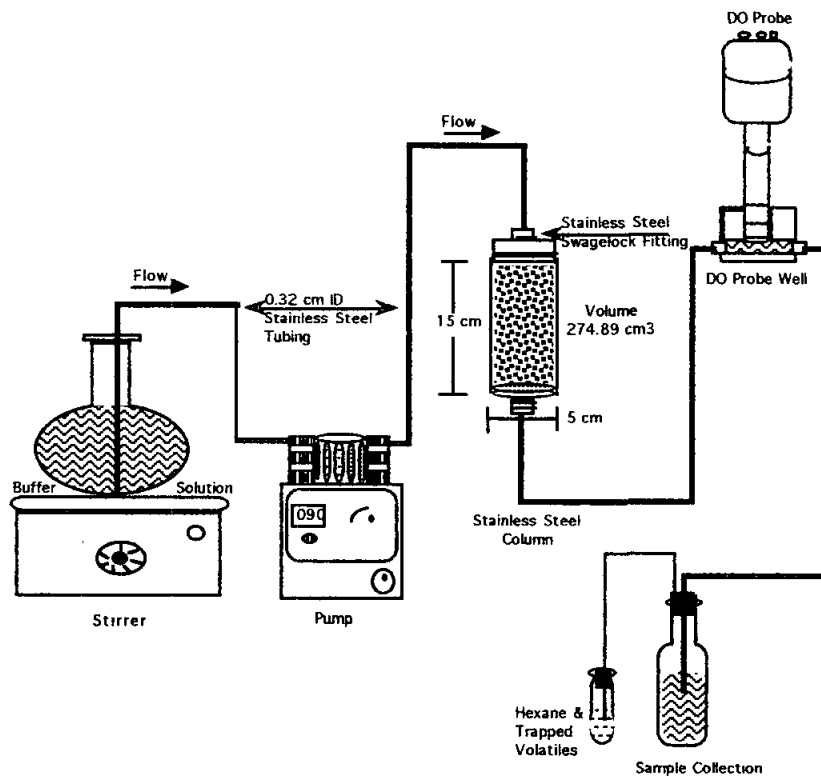


Figure 3.2 Experimental Setup for Column Study

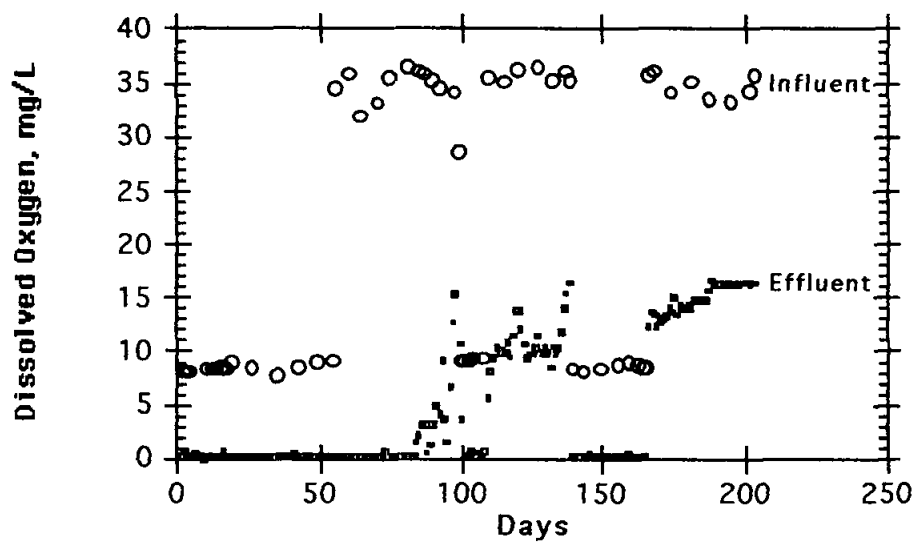


Figure 3.3 Treated Column Influent and Effluent D.O. Profile

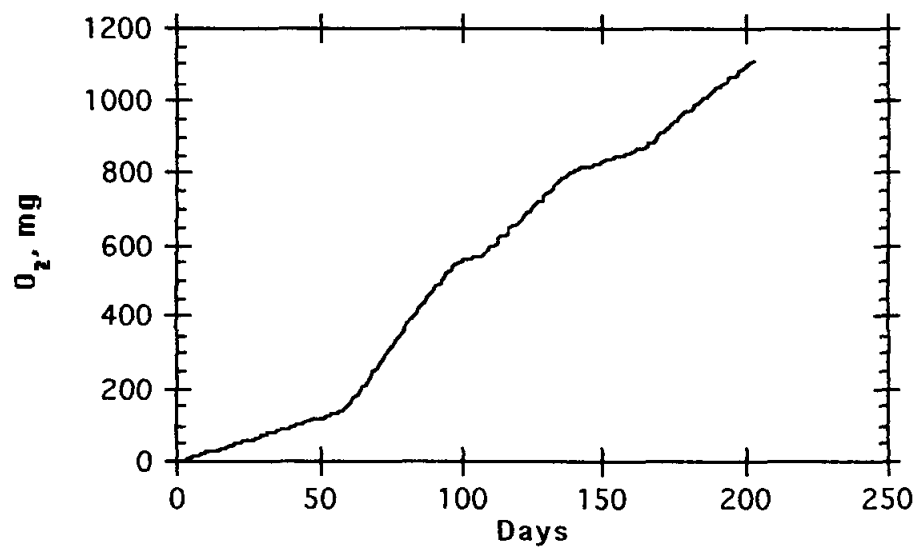


Figure 3.4 Treated Column Cumulative Oxygen Demand

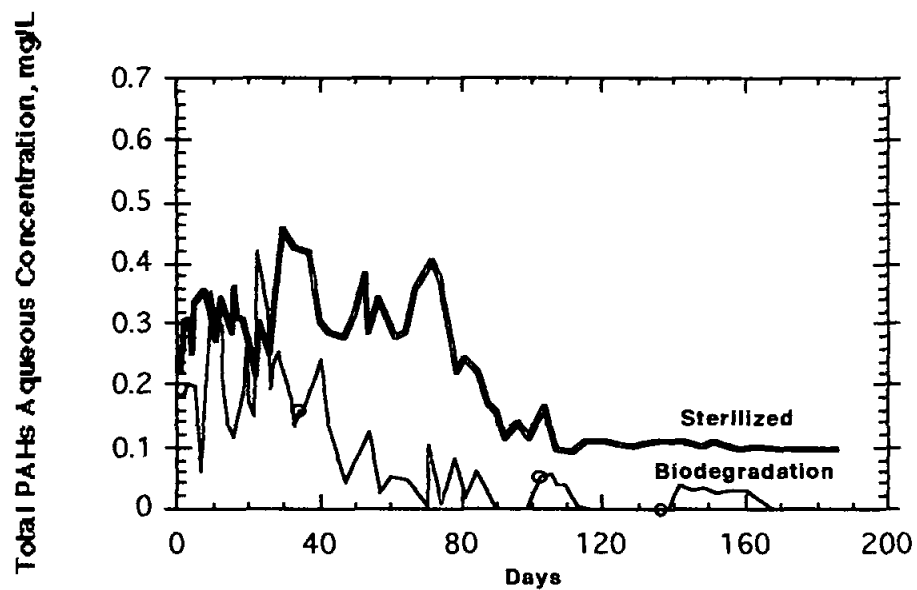


Figure 3.5 Effluent PAH's Concentration Profile

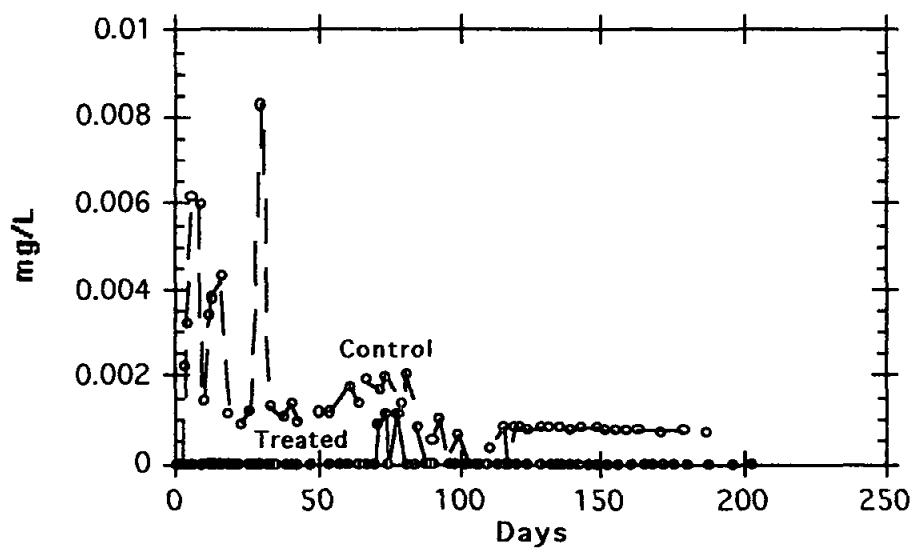


Figure 3.6 Naphthalene Concentration in Column Effluent

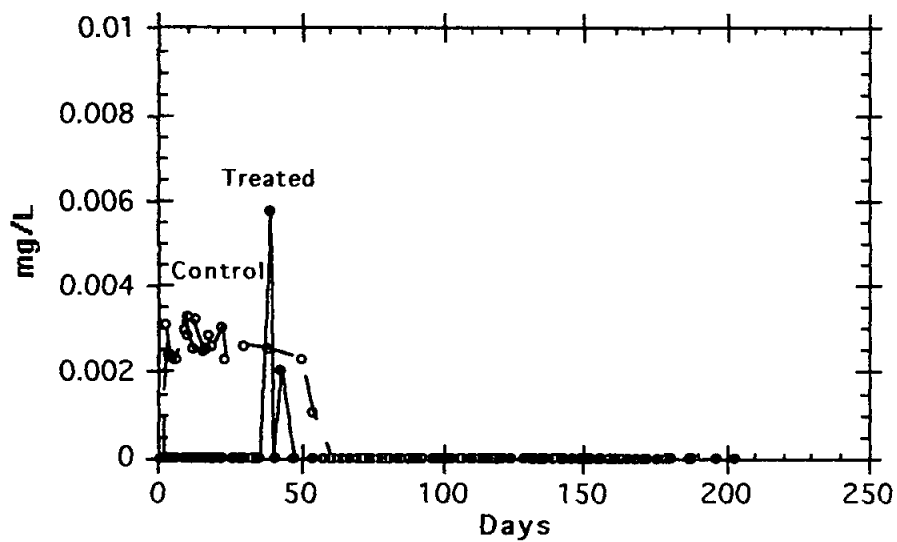


Figure 3.7 Acenaphthylene Concentration in Column Effluent

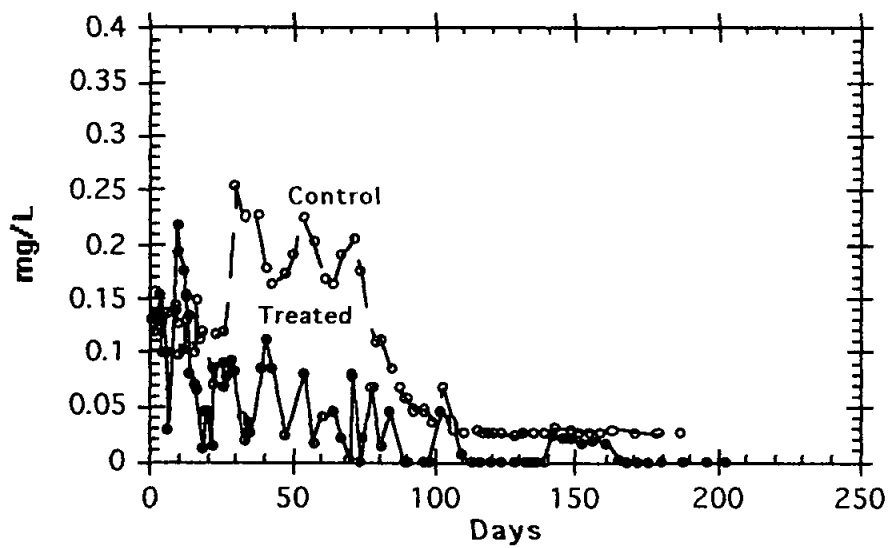


Figure 3.8 Acenaphthene Concentration in Column Effluent

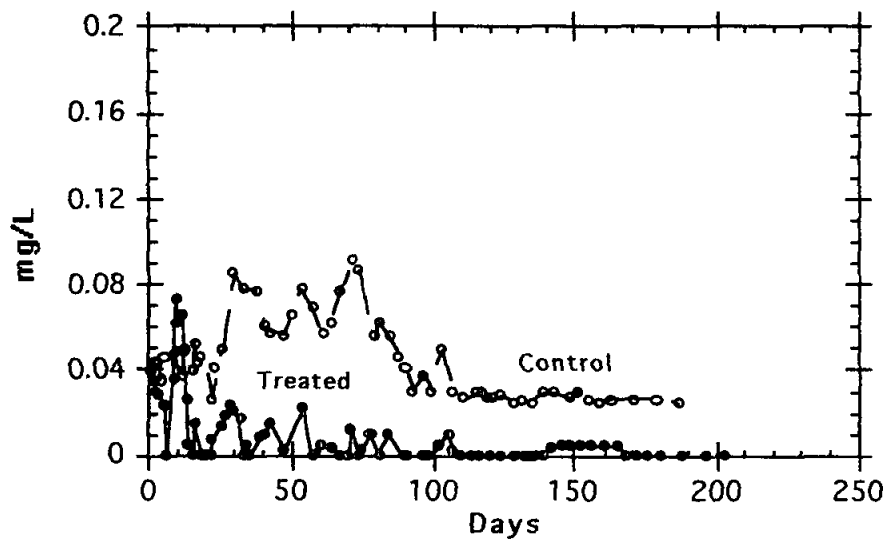


Figure 3.9 Fluorene Concentration in Column Effluent

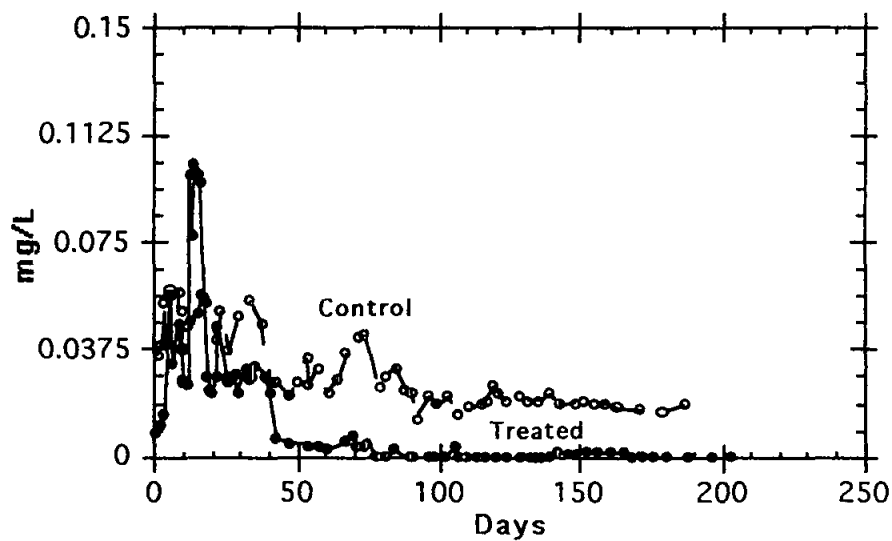


Figure 3.10 Phenanthrene Concentration in Column Effluent

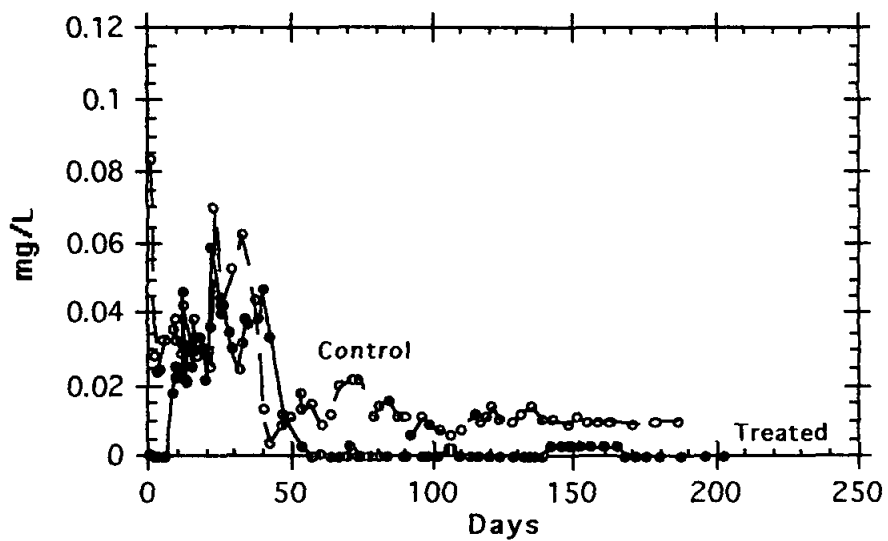


Figure 3.11 Anthracene Concentration in Column Effluent

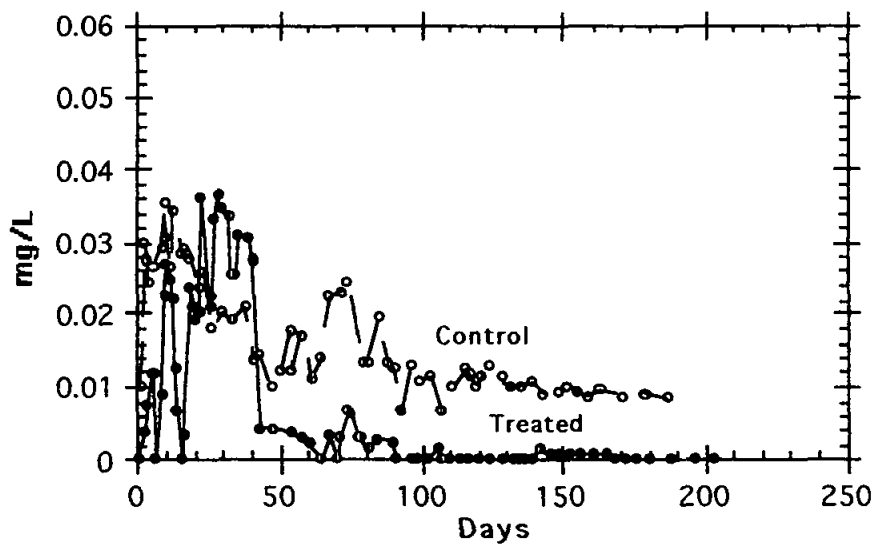


Figure 3.12 Fluoranthrene Concentration in Column Effluent

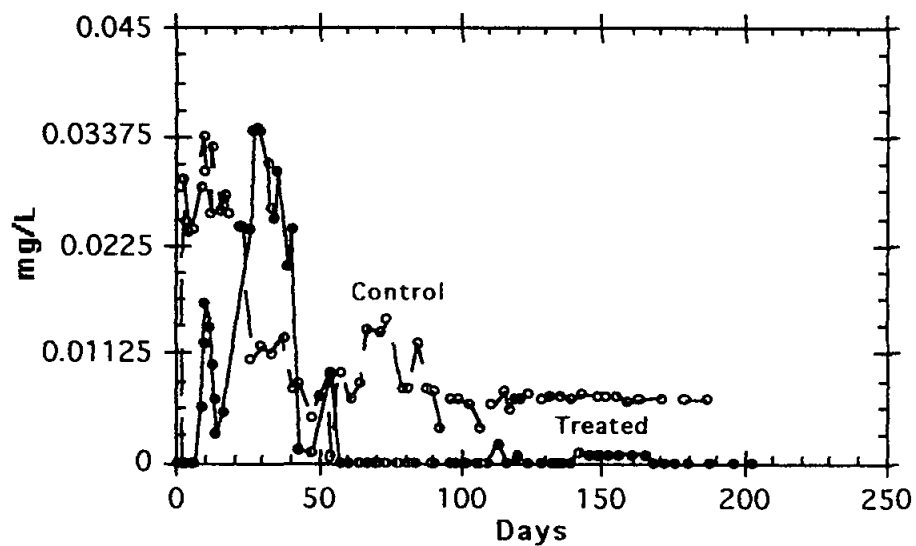


Figure 3.13 Pyrene Concentration in Column Effluent

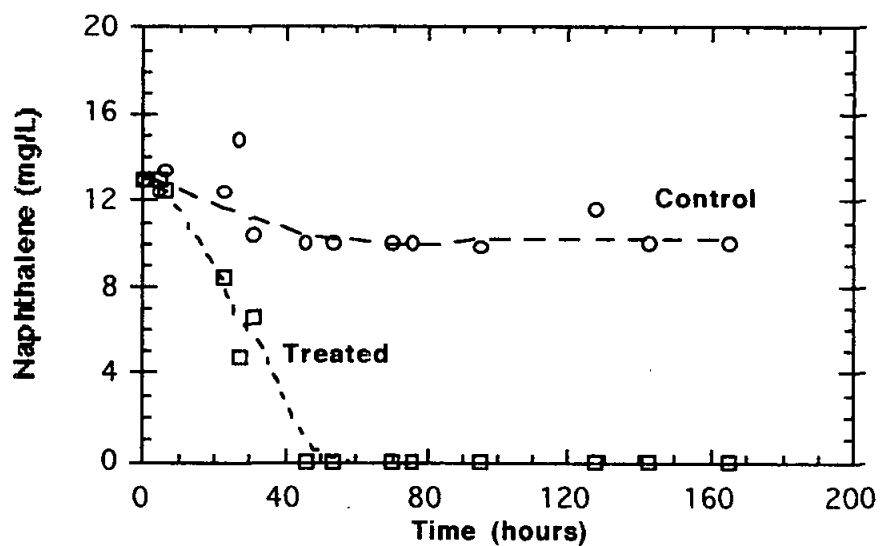


Figure 3.14 Biodegradation Study: Naphthalene Removal Under Aerobic Conditions

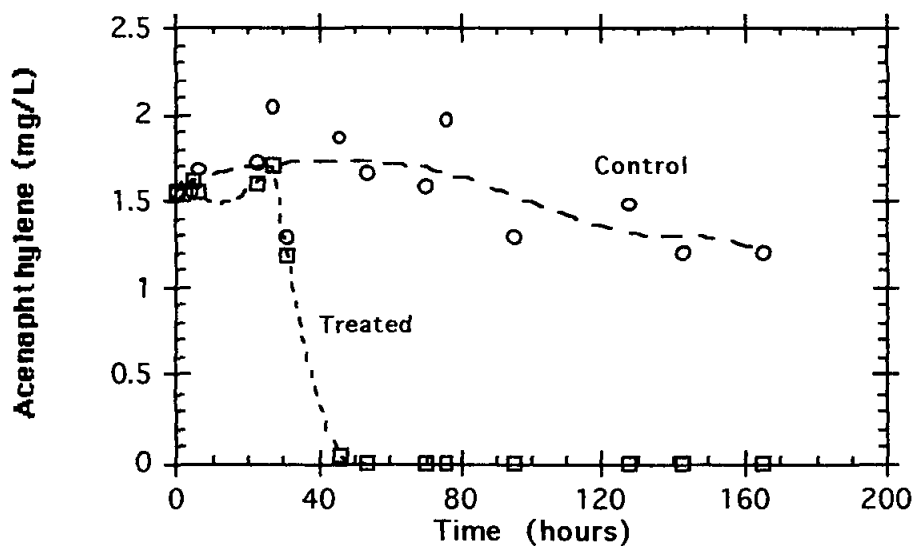


Figure 3.15 Biodegradation Study: Acenaphthylene Removal Under Aerobic Conditions

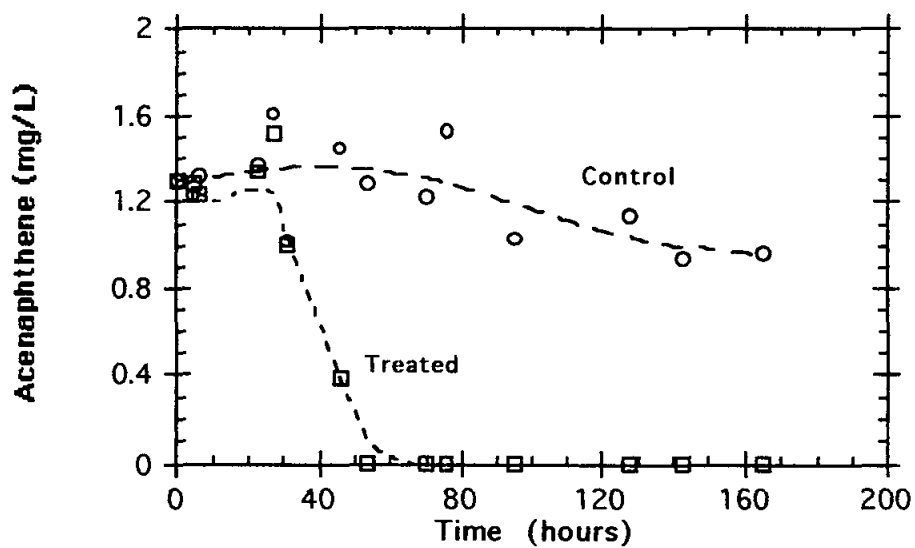


Figure 3.16 Biodegradation Study: Acenaphthene Removal Under Aerobic Conditions

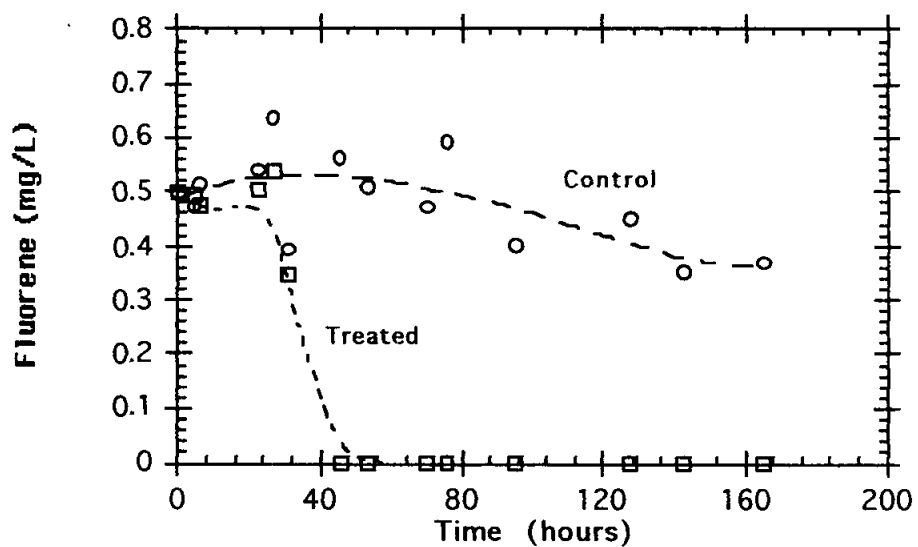


Figure 3.17 Biodegradation Study: Fluorene Removal Under Aerobic Conditions

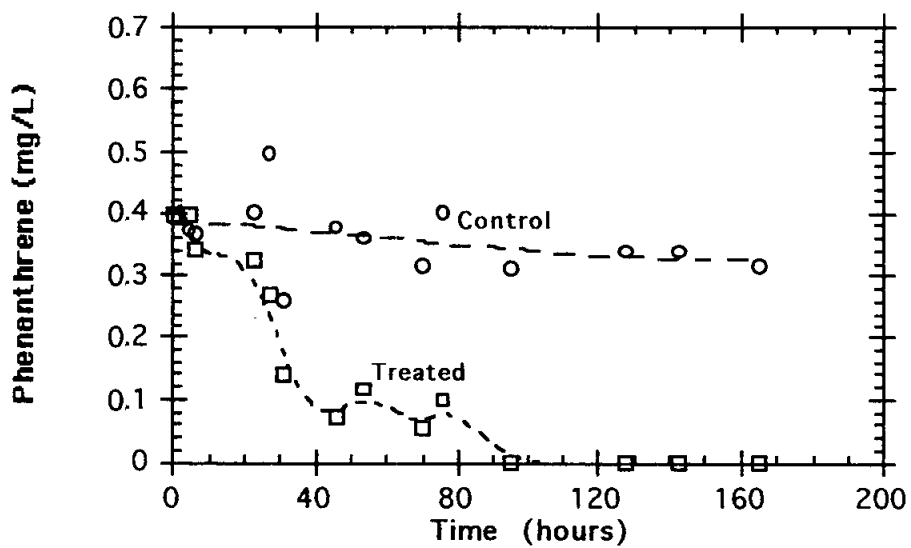


Figure 3.18 Biodegradation Study: Phenanthrene Removal Under Aerobic Conditions

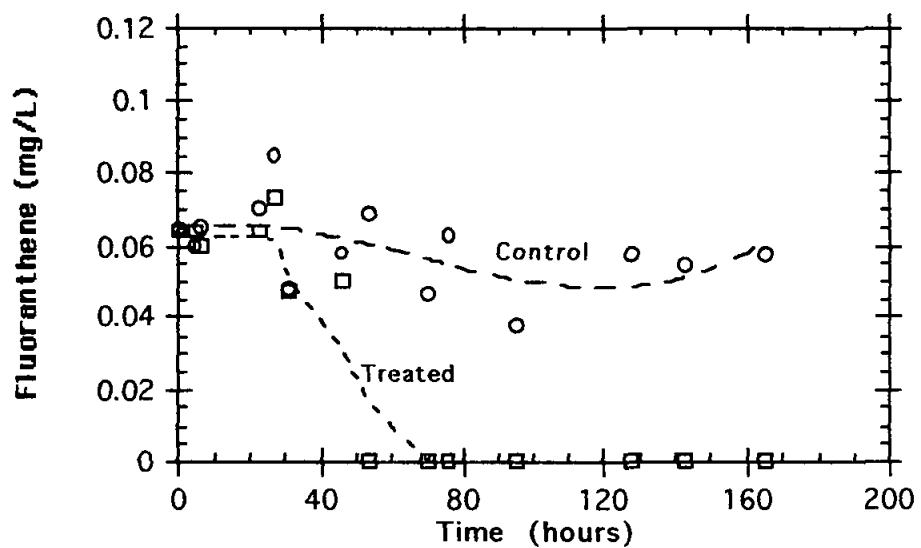


Figure 3.19 Biodegradation Study: Fluoranthene Removal Under Aerobic Conditions

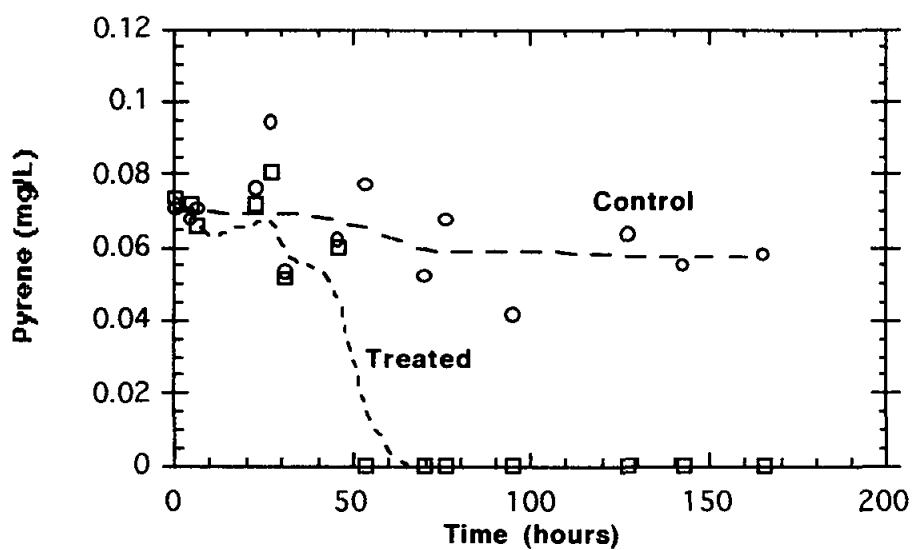


Figure 3.20 Biodegradation Study: Pyrene Removal Under Aerobic Conditions

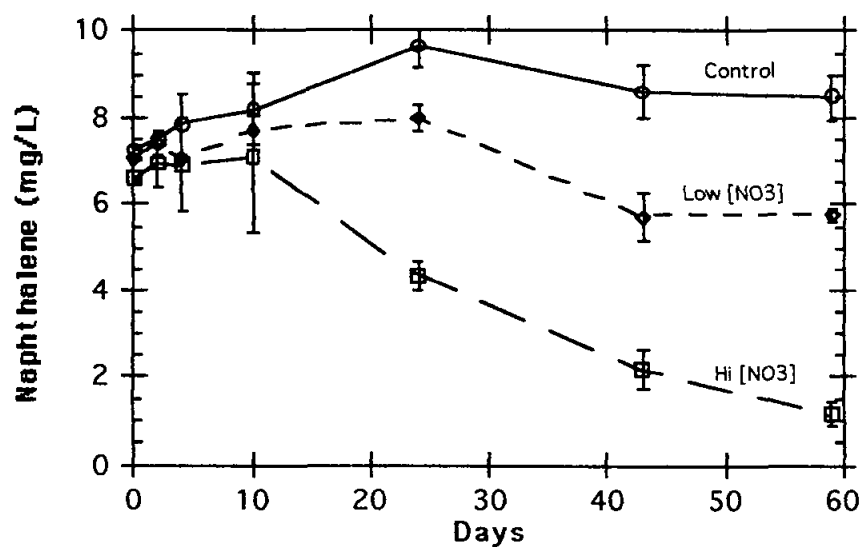


Figure 3.21 Naphthalene Removal Under Denitrification Conditions

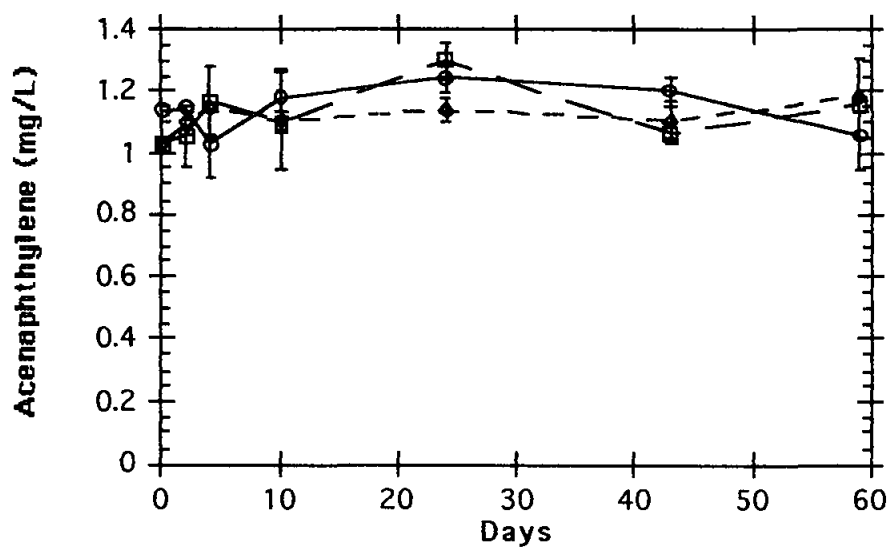


Figure 3.22 Acenaphthylene Removal Under Denitrification Conditions

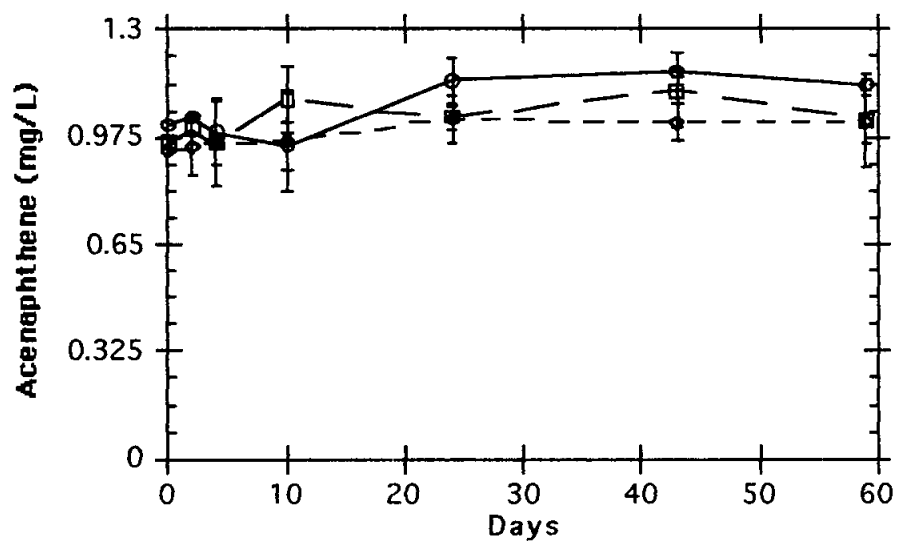


Figure 3.23 Acenaphthene Removal Under Denitrification Conditions

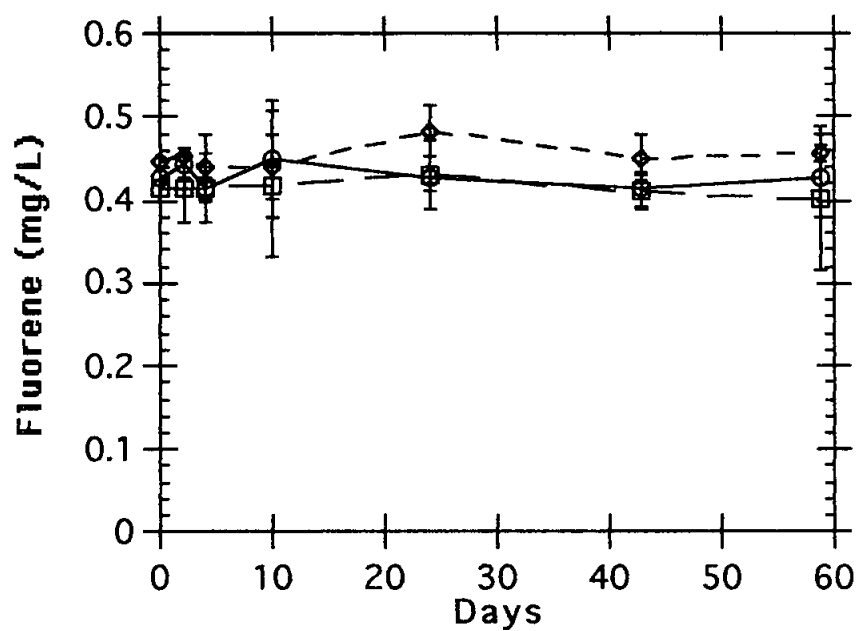


Figure 3.24 Fluorene Removal Under Denitrification Conditions

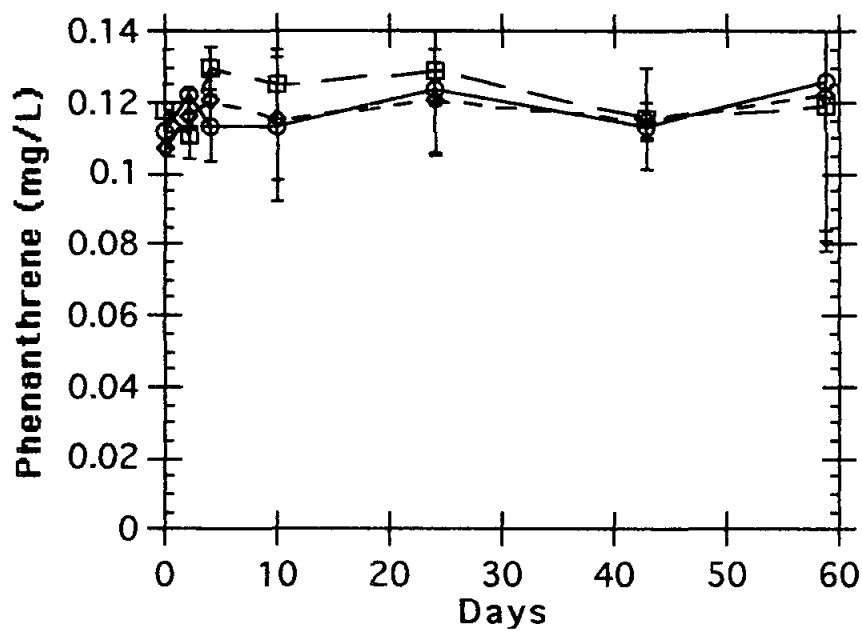


Figure 3.25 Phenanthrene Removal Under Denitrification Conditions

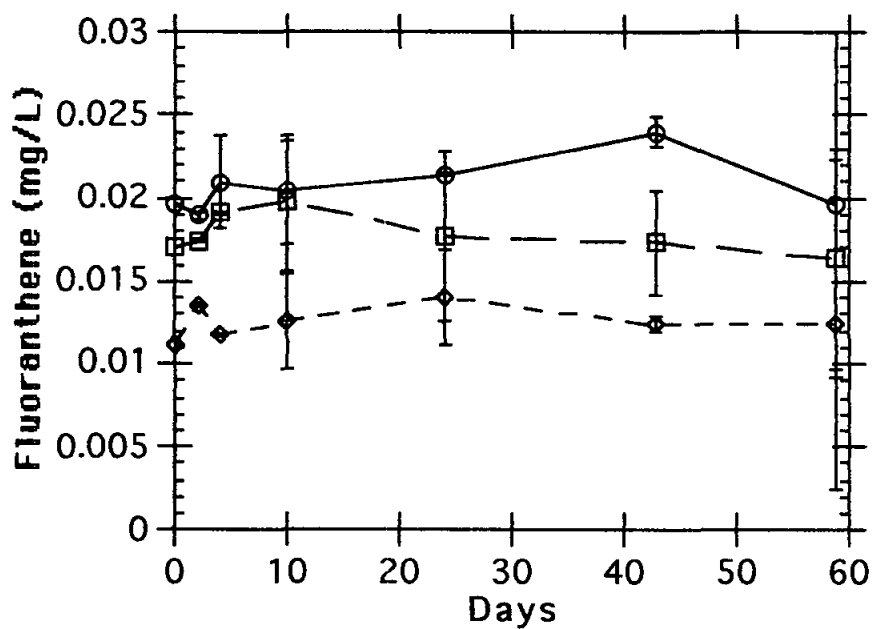


Figure 3.26 Fluoranthene Removal Under Denitrification Conditions

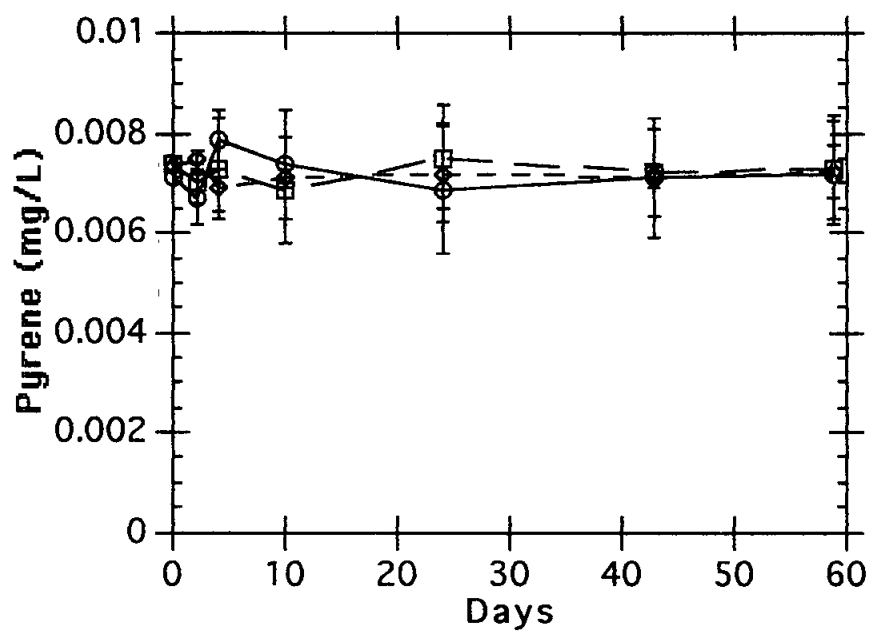


Figure 3.27 Pyrene Removal Under Denitrification Conditions

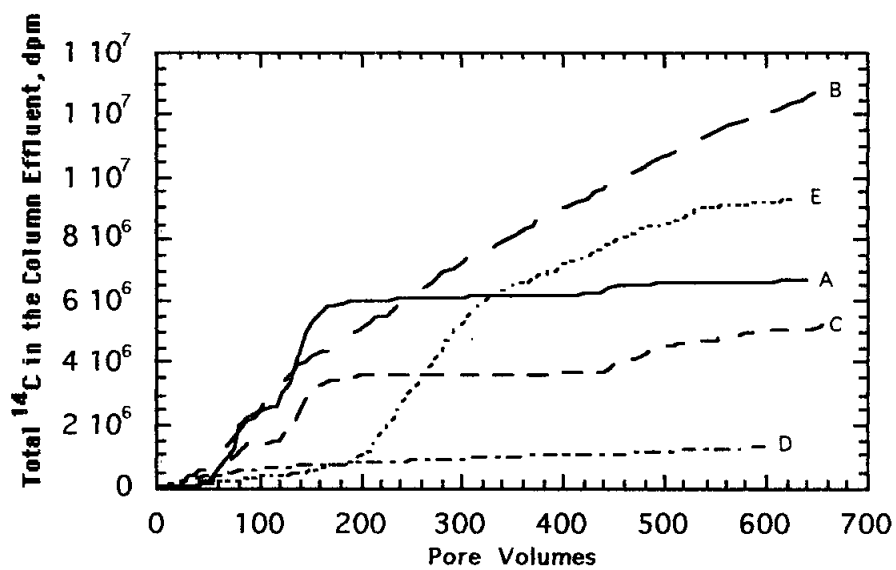


Figure 3.28 Total Radioactivity in the Column Effluent

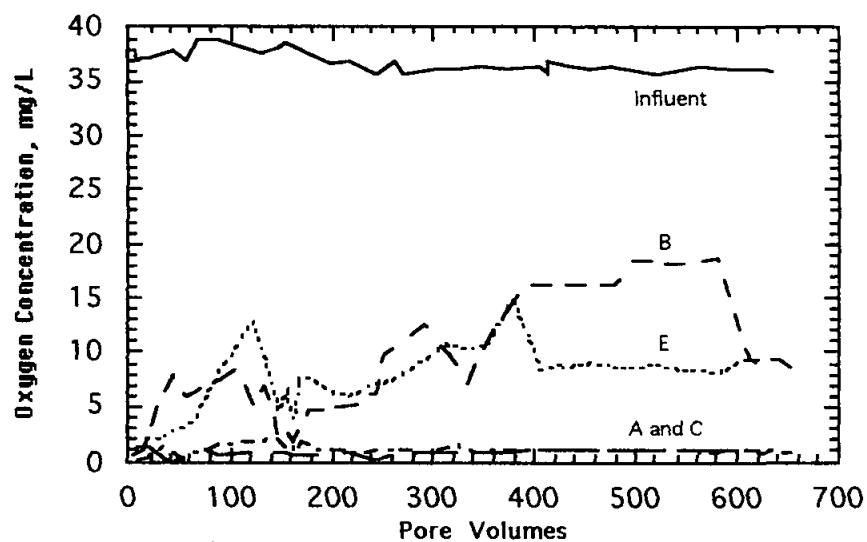


Figure 3.29 Column Influent and Effluent Oxygen Concentration

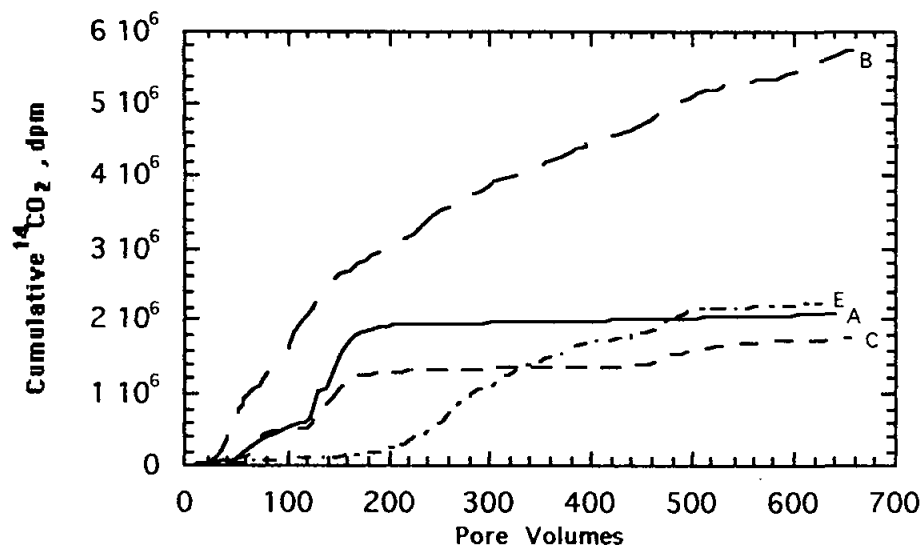


Figure 3.30 Cumulative Radioactive Carbon Dioxide in the Column Effluent

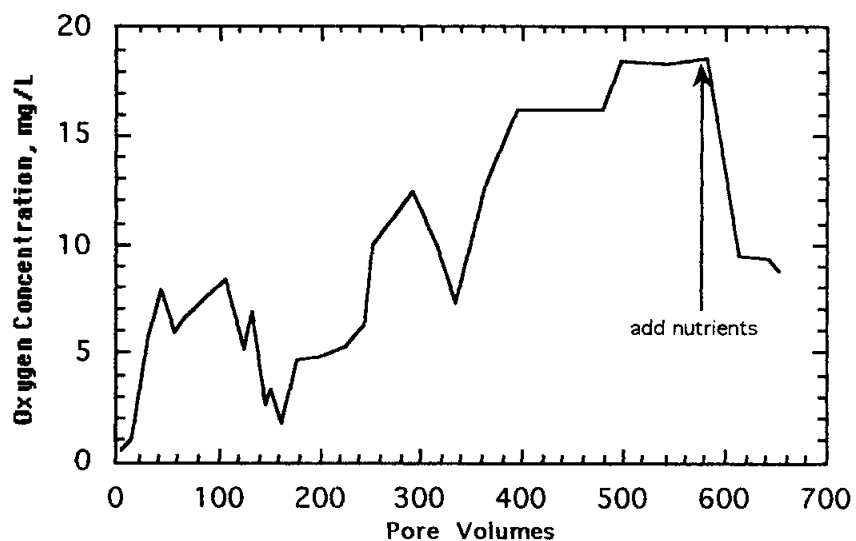


Figure 3.31 Column B Effluent Oxygen Breakthrough Profile

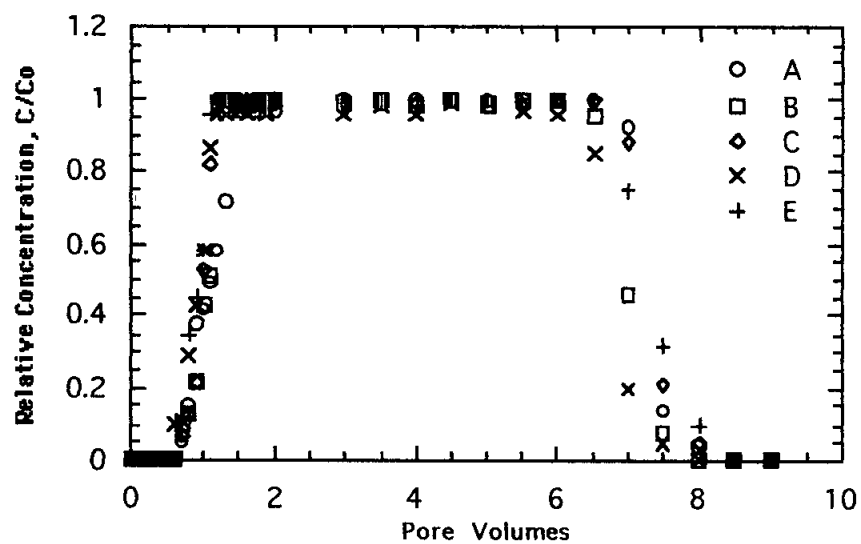


Figure 3.32 Breakthrough Curves of Chloride Tracer for Column Study

Plate 3.1 Column Setup of ^{14}C -Pyrene Biodegradation Study

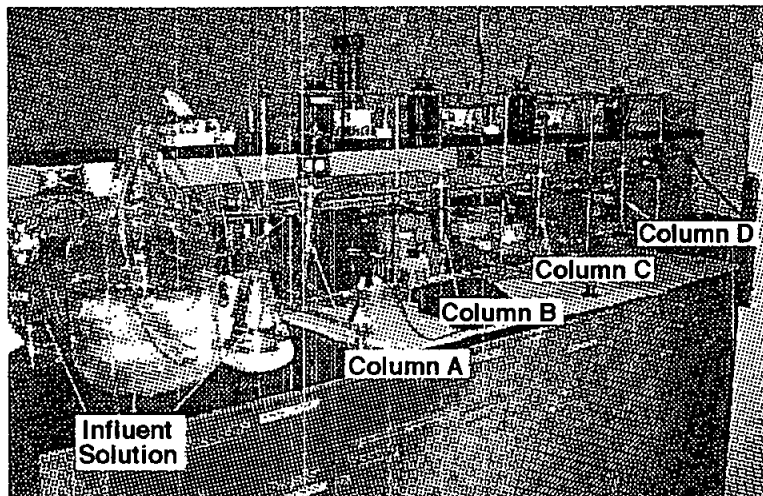
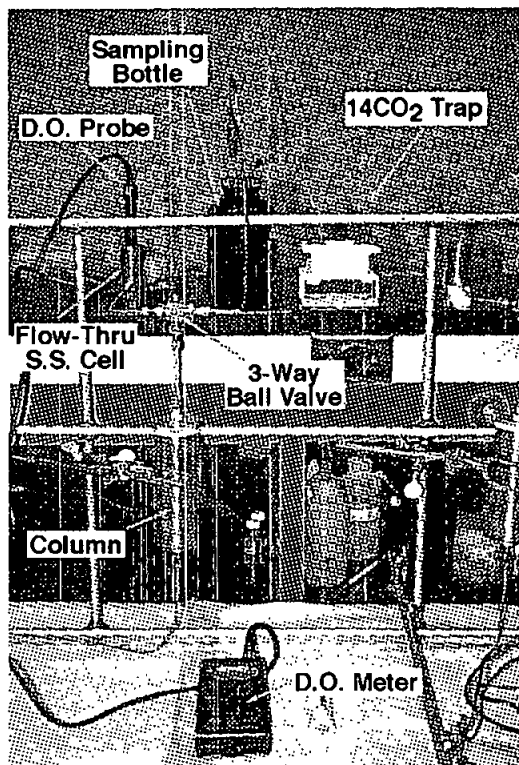


Plate 3.2 Close-up of the Column Setup



Section 4

Split Spoon Insert Column Studies

A. Apparatus for Testing Minimally Disturbed Aquifer Materials

1. SPLIT SPOON INSERT COLUMNS (SSIC)

Development of protocols for obtaining minimally disturbed samples of contaminated soils for testing in the laboratory was a critical initial objective of this research program. Testing of minimally disturbed soils is deemed essential for defining rate data needed to assess the feasibility of insitu cleanup of contaminated aquifers. For this reason, a program for taking cores of aquifer materials by drilling with split spoon samplers with stainless steel inserts and adapting the filled tubes for column studies was undertaken. Needless to say, the procedures could have application at other sites. Construction and operating procedures of Split Spoon Insert Columns (SSIC) are described below.

a. Split Spoon Insert Column Description

The column is the insert from a split spoon sampler containing a minimally disturbed soil core. As shown in Figure 4.1, the column is 24 inches long with an internal diameter of $2\frac{3}{8}$ inches and an external diameter of $2\frac{1}{2}$ inches. The ends of the column are sealed at either end with an aluminum cap. The caps are square blocks of aluminum, 4.5 inches per side with a thickness of $\frac{1}{2}$ inch. A circular groove the same diameter as the column and 0.125 inches wide was cut into the center of aluminum block. A rubber o-ring was inserted in this groove to seal the column to the cap. Three bolts running from the bottom base plate to the top plate secure the base plates to the column. The center of the cap was drilled and tapped to fit an $\frac{1}{8}$ inch stainless steel swagelock fitting.

The water is fed to the column through $\frac{1}{8}$ inch stainless steel tubing. The tubing is connected to the column with $\frac{1}{8}$ inch swagelock stainless steel fittings. The water is pumped through the column using a Gilson Minipuls 2, 4-

channel peristaltic pump. Each column is assigned to one of the four channels. The pump is set up with a 1.52 mm I. D. pump tube with a flow rate of approximately 0.3 ml/min.

The column is equipped with 7 sampling ports located at 3 inch intervals on alternating sides of the column. The ports are constructed from 1/2 inch diameter stainless steel sleeves inserted into holes drilled into the side of the column. The sleeves are sealed to the column with a silicon epoxy. A mini-inert valve is inserted into each sleeve. The valve, as shown in Figure 4.2, is of all Teflon construction and is equipped with a rubber septa which allows insertion of a needle to withdraw water samples from the soil core while maintaining an airtight seal. The mininert valves can be removed to allow removal of soil samples.

b. Core Descriptions

Three soil cores were tested, and are numbered sequentially one to three from the date testing started. The cores were obtained from different depths at the same site located on the ridge running along the western edge of the Reilly site:

Number	Date Started	Depth
1	3/1/92	6'-8'
2	6/3/92	10'-12'
3	6/5/92	4'-6'

The soil in the columns contains a mix of layers of different soil types including silty sand, medium sand, peat and clay. The variety of soil types within a small depth was a result of bulldozing the most heavily contaminated soil into the ridge which runs along the western edge of the Reilly site. The water content of the soil is approximately 10% by weight. The porosity of the soils is 30 % and the average solids density was 2.64 g/cm³.

B. Column Preparation

Drilling to obtain the split spoon insert cores was done on December 11, 1991. The full split spoon inserts were weighed and the ends sealed with plastic caps in the field. The inserts were stored in a temperature controlled room at 10°C until needed. Before testing the split spoon insert was opened and approximately 50 grams of soil was removed from both the top and bottom of the core. This soil was tested to find the physical and chemical characteristics of the soil. A Soxhlet extraction was performed on a 10 g sample of the removed soil to find the concentration of the targeted contaminants in the soil.

After the soil samples are removed the top and bottom of the insert is sealed with a Styrofoam plug and resealed with the plastic cap. The insert was taken to the Civil and Mineral Engineering machine shop. The personnel at the machine shop installed the seven sampling ports and returned the insert to the lab. In the lab the Styrofoam was removed from both ends of the insert. The space between the soil core and the end of the column was filled with washed and sieved Jordan aquifer sand. Two screens were placed on the top of the sand. The first screen is a fine mesh designed to allow water to enter and exit but retain the soil particles. The second screen is a coarse mesh with drainage channels cut into the center. It is designed to direct the water flow to the outflow fitting of the column. The insert is capped on both ends and installed onto a stand.

The water used to saturate the column was deoxygenated to an oxygen content of 0.1-0.5 mg/l by sparging nitrogen into a 5 gallon carboy of deionized water. To begin the flow of water through the column the inlet and outlet tubes are connected to the column to provide up flow. The reverse flow was used to ensure that the soil core was completely saturated. The pump flow rate was set to 0.3 ml/min. and the column was filled until water began to flow from the outlet. Once the flow from the top of the column was established the inlet and outlet tubes were reversed so that the remainder of the experiment was performed with down flow.

C. Sampling Procedures

1. GLASSWARE

All of the glassware was cleaned using the following procedure:

- a. Soap wash
- b. Rinsed and soaked in de-ionized water
- c. Air dried
- d. Rinsed in acetone
- e. Oven dried at 105°C for 24 hours

2. EFFLUENT SAMPLING

The water run through the column is anoxic to prevent biological activity in the column. The oxygen concentration in the water is sufficient to allow free bacteria in the effluent some biodegradation. To inhibit this bacterial growth in the effluent sample it is collected in 500 ml Erlenmeyer flasks which have approximately 0.75g of Sodium Azide added.

D. Effluent Analysis

1. SOLID PHASE EXTRACTION

For the first 136 samples in column one, 39 in column two and 37 in column three a 10 ml aliquot of the sample was removed from the collection flask with a 10 ml gas tight syringe and injected into a 10 ml screw cap test tube. The remainder of the samples had 100 ml extracted. Five μ l of a surrogate spike, 2-Fluorobiphenyl 5000 mg/l in methanol, is injected into the 10 ml or 100 ml aliquot. The spike is added to track the efficiency of the solid phase extraction of the samples. The non-polar organic in the aliquot are extracted and concentrated via a solid phase extraction technique.

a. Equipment:

Varian 500 mg Bond Elute Octadecyl (C-18) cartridges

Varian Vac Elut vacuum source

2 ml class A volumetric test tubes.

Fisher Optima grade hexane

Fisher Optima grade methanol

b. Procedure:

The procedure used is given by Varian for general non-polar isolates. The cartridge is inserted into the vacuum source. First 5 ml of hexane is applied to the cartridge then, one to two ml of methanol is passed through the cartridge to activate the sorbent. The cartridge is immediately rinsed with 1 to 2 ml of deionized water. Immediately after the rinse water has passed the sample is applied to the cartridge. After the sample has passed through the cartridge the cartridge is removed from the vacuum source and allowed to dry overnight.

The non-polar isolates are extracted from the C-18 sorbent by passing a 2-ml aliquot of hexane through the cartridge and collecting it in a 2-ml volumetric test tube. The hexane which is lost due to volatilization during the extraction process is replaced so that the test tube is filled to the 2-ml mark. The final extract is a five-fold concentration of the original sample aliquot.

2. GAS CHROMATOGRAPHIC ANALYSIS

EPA method 8000 (General Gas Chromatography) and EPA method 8100 (GC Analysis of Polynuclear Aromatic Hydrocarbons) were followed.

a. External Standard Calibration:

For each PAH of interest, calibration standards were made using 99.9% pure compounds dissolved in Optima grade hexane (Fisher Scientific). A minimum of five concentration levels were prepared. The concentration levels began near but not below the detection limit of the instrument, the rest of the standards at levels expected of the real samples. The peak areas were compared against the mass injected and a calibration curve was developed for each analyte. A linear fit was prepared for each analyte.

b. GC Analysis Parameters:

Two injections from each sample extract was performed on the GC under the following conditions

GC . Hewlett Packard 5890 with auto sampler	
Integrator	HP 3396
Column	HP-5, 25m x 0.2 mm x .33 μ m
Detector	FID
Carrier Gas	Hydrogen, 2ml/min
Initial Temp	40°C
Initial Time	1 min.
Rate	6°C/min
Final Temp	2°C
Final Time	8 min
Injection vol	2 μ l

E. Intermediate Column Samples

Intermediate samples were taken along the length of the column through the sampling ports. These sample were taken about every other week.

1. PORT SAMPLING METHOD

To sample the ports a 10-ml syringe, with the plunger removed and a 4-inch, 22 gauge needle was used to penetrate the septum of the mininert valve in the sampling port. Once the septum was penetrated and a flow into the syringes was established the syringe was clamped to a stand and allowed to fill. The syringe filled by the flow from the port alone the plunger was not used to draw out a sample. This was done to prevent desaturating the column and to provide a more representative sample of the column water at the position of the port.

The ports were sampled sequentially from the bottom of the column to the top. Each sample takes about 20-30 minutes to collect.

2. ANALYSIS

The samples from the ports were extracted and analyzed using the same methods as the effluent samples.

F. Column Soil Samples

The soils from the columns were sampled and analyzed on three separate occasions.

1. INITIAL SOIL SAMPLING

During column preparation soil samples from the top and bottom of the soil core were taken. These samples were combined and mixed to form a composite. This soil was then analyzed, via soxhlet extraction and GC analysis to get a rough estimate of the soil PAH contamination.

2. INTERMEDIATE PORT SAMPLING

This sampling was done after the anoxic testing stage before any oxygen was added to the column. The purpose of the sampling was to determine the PAH distribution in the soil along the length of the column and to get a base line to measure the effectiveness of bioremediation on removal of PAH's from the soil phase.

This sampling was done by removing the mininert valves from the column at depths of 6, 12 and 18 inches from the top of the column. Once the valve was removed a 3 to 5 gram sample of the soil was withdrawn from the core. The void created by removing the sample was filled with sand from the Jordan aquifer to avoid serious alteration of the column hydraulics. The soil sample was then analyzed via soxhlet extraction and GC analysis to find the level of PAH contamination and fraction of organic carbon (foc).

3. FINAL SOIL ANALYSIS.

The final soil analysis was done at the conclusion of the column experiments. The soil columns were sacrificed. The soil was removed from the columns in 3 inch increments. The soil was classified by physical appearance, weighed and placed in air-tight sample jars. The soil samples were analyzed for PAH contamination levels and via Soxhlet extraction and GC analysis.

G. Soxhlet Extraction and Analysis

The Soxhlet Extraction procedures described under EPA method 3540 was followed. The method is for extraction of nonvolatile and semivolatile organic compounds from solids, sludges, and wastes, and is applicable to the isolation and concentration of water-insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures. This procedure is given earlier in this report.

1. GAS CHROMATOGRAPH ANALYSIS

Gas Chromatograph analysis of the soils was done as with the SPE extracts given previously.

H. SSIC Test Results

1. GENERAL OPERATIONS OF THE SPLIT SPOON INSERT COLUMNS (SSIC)

Three split spoon insert column soil cores from the Reilly site are currently being tested to measure rates of removal of creosote related organic chemicals. The apparatus is shown in Plate 4.1.

a. Initial Operations

The columns were dosed continuously with pure deionized water. The flow rate and oxygen content of the water was varied to determine the effect those parameters had on the dissolution and aqueous concentrations of the PAH's. A description of the various conditions of flow and the results of each condition of flow will be detailed in the following sections.

Column #1 has been in operation for over 12000 hours with continuous dosing. Columns #2 and #3 have been in operation for over 10000 hours. During this period, samples of water from the outlet and at intermediate sampling port have been taken periodically and analyzed using an adaptation of EPA prescribed

analytical procedures for concentrating and measuring PAH's by GC analysis as described in the previous section.

In column one effluent concentrations of four PAH's, naphthalene, acenaphthene, fluorene and phenanthrene were monitored during the first 700 hours of dosing are shown in Figures 4.3-4.14. The day to day variations in concentration were larger than expected and it was found that most of these variations were due to experimental problems that have since been eliminated. The causes of these problems and their solution are briefly summarized to show that the SSIC testing protocol is reliable but requires attention to operating procedures, particularly with regard to access to molecular oxygen and handling of samples prior to analysis.

One problem was caused by diffusion of oxygen from the air through the teflon tubing that was used to pump deoxygenated water to the column. It had been decided to initiate the test by using anoxic conditions to test removal of organics by flushing without biodegradation. As a result of the unexpected availability of oxygen the initial results from column one were not useable and had to be discarded. Teflon tubing was replaced with stainless steel, eliminating this source of oxygen. The second problem resulted from biodegradation of organics in the collection vessel caused by the presence of microbial cells and oxygen. This problem was solved by adding sodium azide to the collected effluent to stop microbial activity. It should be noted that the observed biological removal of organics is actually desirable from a practical viewpoint because it is evidence that microbial activity is capable of removing the target chemicals. However, for this first phase of the study, the objective was to measure the rates of elution with minimal biological activity. This type of information is needed to quantify the physical rates of removal, namely desorption and dissolution and subsequent transport of the solubilized species through the rest of the column.

b. Stabilized Operations

1. *Anoxic Conditions*

The purpose of this portion of the experiment was to determine the long term dissolution behavior of the PAH's with no biotransformation. After solving the problems described above the columns were dosed with deionized water which was deoxygenated at a flow rate of 0.3 ml/ min. This flow rate corresponds to a Darcy velocity of 1.75×10^{-4} cm/s and a pore velocity of 5.83×10^{-4} cm/s. These conditions were maintained from day 35 to day 184 in column one, day 1 to day 144 in column two and day 1 to day 142 in column Three.

The leaching of the four monitored PAH's follows a pattern which can be generalized to all four PAH's and all three columns. The effluent concentrations peak at or near the start of column operation and gradually decrease to a stable value.

Naphthalene had the highest effluent concentration in columns one and three. The naphthalene concentration in these columns peaked at about 1.0 mg/l and then dropped to a steady state concentration of approximately .05 in column one and 0.3 mg/l in column three. The naphthalene concentration in column two was very low from the very beginning and never reached a well defined peak. The effluent concentration from column two was fairly constant ranging in value from .01 to .015 mg/l during the time of this portion of the experiment. None of the concentrations reached a value close to the saturation concentration of 31.7 mg/l.

Acenaphthene leaching was similar in all three columns. In each column the acenaphthene reached a peak value of between 0.15 and 0.20 mg/l. The concentrations then dropped to values between 0.06 to 0.09 mg/l and remained fairly steady with very little variation in concentrations. The peak concentrations did not approach the acenaphthene saturation concentration of 3.93 mg/l.

The fluorene leaching was more variable between the three columns. In column one the effluent concentration reached a peak of 0.2 mg/l after approximately 80 days of operation. The concentration then fell to a steady concentration of 0.06 mg/l after approximately 150 days. In column two a peak concentration of 0.078 mg/l was reached after approximately 70 days

of operation. The effluent in column two did not drop significantly, dropping only 0.02 to 0.04 mg/l after 130 days. In column three the fluorene concentration peaked at 0.1 mg/l after 35 days of operation then gradually decreased to a low of 0.04 mg/l after 130 days of operation. As with naphthalene and acenaphthene the fluorene concentration was not ever near the saturation concentration of 1.98 mg/l.

The phenanthrene effluent concentration also showed some variation between the three columns. In column one the phenanthrene concentrations vary from .03 to 0.10 mg/l. The initial leaching shows relatively steady concentrations of approximately 0.035 mg/l for the first 110 days. The concentration then forms a hump where the effluent slowly builds to 0.1 mg/l from 110 to 140 days and then drops to 0.05 mg/l at 160 days. The phenanthrene in the effluent of column two over the first 50 days shows a large amount of variation, from 0 to 0.07 back down to 0.02 mg/l in the first 20 days of operation. The concentration then rises to and remains fairly constant at 0.03 to 0.04 mg/l. In column three the effluent concentration varied from a high of 0.1 to 0.025 mg/l over the first 60 days. The concentration became fairly constant after 110 days at 0.03 to 0.04 mg/l.

The effluent concentrations of the monitored PAH's never approached the aqueous saturation values of these compounds. This indicates that the effluent concentration is controlled by other factors. The other factors which influence the effluent concentration include, dissolution rates between an immobile boundary layer between the moving water and soil particles, desorption from the organic carbon component of the soil and the Raoult's law.

Conclusions

1. This test has given us a large data base of the relative leaching rates of specific compounds.
2. Rates of leaching are slow but significant. The low concentration of PAH's in solution, none approaching solubility, suggests that dissolution is strongly influenced by desorption from the organic carbon

component of the soil and Raoult's law of dissolution of mixed compounds.

3. The leaching is at rate which mobilizes significant amounts of PAH's is capable of contaminating a large volume of water yet is slow enough that the source removal will take on the order of hundreds of years.
4. The general leaching pattern of concentrations reaching a relative maximum then decreasing relatively rapidly to a stable concentration suggests that DNAPL is present. This free product contributes to the initial high concentrations and, as it is exhausted, the concentration falls reaching a pseudo-equilibrium. This characteristic may give false treatment time estimates and should not be overlooked when doing field studies.
5. The effective modeling of these leaching rates is hampered by the complexity of the system and by the extreme heterogenous nature of the soil and contamination concentrations.

2. Ambient Oxygen Concentration

The purpose of this portion of the experiment is to determine if ambient oxygen concentrations are sufficient to oxygenate the entire length of the column and to observe the effects of biodegradation on effluent concentrations of the PAH's. The water fed into the columns was allowed to equilibrate with the atmospheric oxygen resulting in an influent oxygen concentration of approximately 8 mg/l. All other operating parameters remained the same. These conditions were maintained from day 185 to day 282 in column one, day 145 to day 198 in column two and day 143 to 196 in column three.

The effluent concentrations of the monitored PAH's were not significantly affected by the addition of low amount of oxygen. The effluent concentrations dropped slightly in some instances over the first ten days of oxygen addition but then most recovered to the concentrations recorded prior to oxygen addition. These concentrations remained fairly constant

through the remainder of the time the columns were run under these conditions.

Conclusions

1. The addition of insufficient oxygen to ensure adequate oxygen over the entire column length does not halt the mobilization of PAH's. The oxygen is exhausted in the first few inches of the soil column, the remainder of the column leaches enough PAH's to replace any removed by biodegradation.
2. As a result any large scale remediation effort must ensure adequate oxygen to avoid excessive off site migration of targeted contaminants.

3. *Ambient Oxygen with Varying Flow Rates*

The purpose of this portion of the experiment was to investigate the effect of flow rate on the dissolution of PAH's into aqueous solution. And to gain more insight into the controlling mechanisms of the PAH dissolution. This was done by running the columns at three different flow rates. The first flow rate was the lowest, with an average of 0.130 ml/min. This flow rate corresponds to a darcy velocity of 7.6×10^{-5} cm/s and an average pore velocity of 2.5×10^{-4} cm/s. The second flow rate was identical to the one used in the anoxic and ambient oxygen conditions. The final flow rate was highest with an average of .510 ml/min, corresponding to a darcy velocity of 2.98×10^{-4} cm/s and an average pore velocity of 9.91×10^{-4} cm/s. The timing and duration of the three flow rates is summarized in Table 4.1.

The effect of varying the flow rate is summarized in Figures 4.15-4.26 and in Table 4.2.

The results show that the effluent concentrations decrease with increasing flow rates. The only exception is the phenanthrene concentration increases slightly when the flow rate is increased from low to medium. This inverse correlation between flow rate is misleading, it suggests that the rate of dissolution decreases with increasing flow rate. This is not true. The overall

removal rate, weight of contaminant removed per unit time, in most cases is increasing with the increase in flow rate.

The exceptions, where the flow rate increase causes a decrease in removal rate, suggest that there is an optimum flow rate which maximizes the removal.

Conclusions:

1. The increase in flow rate, in general, increases the rate of dissolution. This suggests that the overall leaching rate is controlled by the diffusion of the PAHs from a immobile film of water which surrounds the soil particles into the bulk, mobile water. As the flow rate is increased the thickness of this film decreases allowing higher dissolution rates.
2. This effect is limited. There is an optimum flow rate which maximizes dissolution - higher or lower flow rates will have less than optimum dissolution. This optimum flow is determined by the complex interaction of boundary layer diffusion, partitioning rate, and concentration of PAH's in the bulk water, immobile film and sorbed to the soil.
3. Any mathematical dissolution model will have account for this effect to offer accurate analysis.
4. Field test should be conducted before full scale treatment to determine optimum flow rates.

4. *Saturated Oxygen Concentration and Varying Flow Rate*

The purpose of this portion of the experiment is to determine the flow rate that would allow oxygen breakthrough and to determine the effects of sparging with pure oxygen on the PAH dissolution and effluent concentrations. The influent deionized water was sparged with pure oxygen to achieve dissolved oxygen concentrations of 35 to 40 mg/l. The initial average inflow rate was .280 ml/min. This rate was maintained for 26 days

without any breakthrough of oxygen in the effluent. The flow rate was increased to an average of .570 ml/min. At this flow rate there was oxygen breakthrough in columns one and two but not in column three. The operating parameters are summarized in Table 4.3.

The effect of the pure oxygen on the effluent concentrations is shown graphically in Figures 4.27-4.38. The graphs show that the initial effect of the increased oxygen is minimal at the lower flow rates. In all three columns and all four monitored PAH's showed very little change in effluent concentration. This changes, however, with the increased flow rate. At the increased flow rate the effluent concentrations of all of the PAH's drops rapidly in all three columns. Simultaneously, with the exception of column three, the effluent oxygen concentration begins to increase. In columns one and two, after significant oxygen breakthrough, the concentration of the monitored PAH's dropped to non detectable levels. There was no significant oxygen breakthrough in column three and there was always detectable levels of PAH's though they did drop off rapidly with the increased flow rate.

Conclusions:

1. If sufficient oxygen is available throughout the column the PAH concentrations are reduced to non detectable levels. This suggests that the biodegradation of the targeted compound is limited to the availability of molecular oxygen.
2. The oxygen demand of the column is approximately 30 mg/24 hr/2000cm³ soil. This suggests that this amount of oxygen must be supplied to the treated soil to expect sufficient oxygen to prevent off site migration. Field tests should be done to confirm this approximation.
3. No nutrient addition was required to achieve biodegradation of mobilized PAH's. This must be confirmed with pilot scale field tests.

c. Concentration Profiles at Intermediate Points

As indicated in the procedures section, water samples were taken from each of the intermediate sampling ports in order to measure concentration distributions at selected time intervals. The depth measurement is given with reference to the top of the column. The results of the intermediate sampling ports are summarized in Figures 4.39-4.41

The results show some interesting trends. In all three columns the concentrations increase in the downstream direction and then decrease in the lower half of the column. Increases in concentration in the downstream direction were not unexpected because of the cumulative contact time between water and soil phases. However, the downward concentration trends in the lower half of the column are due to other causes. One possibility is that there was non uniform distribution of chemicals in the bed initially. Another possibility is that continuous leaching of the column has resulted in a substantial concentration gradient in soil organic content (foc). This could result in readsorption of chemicals in those parts of the bed with the highest foc.

The intermediate sampling during the saturated oxygen conditions also shows some interesting results. In column one, for example, the intermediate ports show some significant concentrations of PAH's where the effluent concentrations have dropped to below detectable levels. Column two is similar, for the sample taken on day 413 there is detectable levels of PAH's at depths of 18 and 21 inches but the effluent concentration on that day was non detectable. These results suggest that the dissolution rate is faster than biodegradation rates in certain portions of the column. The biodegradation is sufficient, however, remove the PAH's before they reach the collection vessel as they pass through the packed sand at the end of the column and the plumbing of the column.

Conclusion:

1. Concentration profiles (snapshots in time) show some expected as well as unexpected results.
2. Present models are inadequate to give the same dissolution patterns.

3. Dissolution rates are sufficient, in some instances, to show PAH's in solution despite the presence of oxygen. This may indicate that the biodegradation rate is slower than dissolution or that there are areas of the column which are locally anoxic.

2. ANALYSIS OF SOILS FROM COLUMNS

a. Initial Soil Analysis

The soil for this analysis was obtained from the top and bottom of the columns before any treatment had occurred. The results of this analysis are summarized in the Table 4.4. These results give a limited indication of the relative soil concentrations of the various PAH's. Since the samples were limited to only the top and bottom of the column it is not advisable to apply the results to the entire column.

b. Intermediate Point Samples

The results of analysis of the intermediate sample removed from the sample ports during column operation are summarized in the following Table 4.5. The results of the analysis are mixed. They show that the PAH contamination in the cores is very spatially variable. In column three the phenanthrene contamination varies from 42.5 to 1097.1 mg/kg in a space of 6 inches. When compared to the aqueous samples taken from the ports it shows that where the soil content of PAH's is low the dissolve concentration is also low. The aqueous concentration in column two is consistently higher in the samples taken from a depth of 18 inches, the soil analysis shows that is also the position of the highest soil concentrations. This also hold true at the 12 inch depth in column two. This suggests that there may be some dynamic equilibrium where the dissolution of the PAH's occurs in the highly contaminated soils with some readsorption in the less contaminated soils.

The soil samples taken from the ports were also analyzed to determine the fraction of organic carbon (foc). The organic carbon content plays an important role in the adsorption of organics in soil systems. In general the higher the foc would indicate higher contamination levels due to the adsorption of the PAH's

to the organic carbon fraction of the soil. These results show that there is little correlation between foc and the level of PAH contamination. This data is a result of analysis of very small soil sample and may be misleading.

c. Final Soil Analysis

The results the analysis of the soils which were done at the conclusion of the column experiments is summarized in Figures 4.42-4.44. These results show the variability of the soil contamination. The contamination level varies by as much as three fold over just three inches of depth. This variation makes it difficult to model dissolution or biodegradation rates. It will also complicate any full scale remediation, care will have to be taken during soil sampling to avoid leaving localized hot spots under treated.

In addition, the relative amounts of the individual PAH's present in the soil after treatment show some interesting trends. The more soluble compounds are have relatively low soil concentration throughout the column. For example, naphthalene concentrations are very low through out all three columns but the concentrations of fluoranthene are fairly high. This is not unexpected since these compounds are more mobile and, as a result, their rates of removal are higher then the less soluble materials. This requires that the design of any treatment system should be based on the rate of removal of the less soluble compounds to avoid inadequate treatment of the the soils.

3. MASS BALANCE ANALYSIS

A mass balance approach to the removal of the four monitored PAH's from the three column was done to provide some insight into the relative removals attained by both biodegradation and leaching. This analysis is based on several assumptions:

- a. The only mode of removal during the anoxic phase of the experiment was by leaching.
- b. The oxygen content of the influent water during the ambient oxygen and varying flow rate conditions was constant at 8 mg/l.

- c. The biodegradation of total PAH's from the column was related to oxygen consumption by a ratio of 7 to 1, every milligram of PAH removed required 7 milligrams of oxygen. This ratio was obtained experimentally from experiments performed on the composite soil column.
- d. The percent removed is based on the sum of the weight in soil after treatment, leached amount and the amount biodegraded

The results of the analysis are given in Table 4.6. The results clearly show that the rate of leaching is very slow. The columns were in operation for approximately 1.5 years and only a very small percentage (average of 15 % of the total removal amount) is from the leaching of the contaminants. In contrast the estimated amount biodegraded is substantial, accounting for 88% of the total removal. The majority of the biodegradation occurred during the saturated oxygen flow conditions which only lasted about 3 months.

The total percent of the contamination removed varied from 55% to 27%. This was largely a function of the initial concentrations present in the column. Since the columns were all run under the same conditions this is not surprising. However, a consequence is that the treatment time must be geared to the highest soil concentration. Failure accurately define the contamination levels may lead to inadequate treatment.

The estimated soil concentrations from the initial soil analysis completed before any treatment of the columns was not very accurate. When compared to the mass balance amounts calculated after sacrificing the columns only column one was close the other two were off by 71% to 94%.

Conclusions

1. Leaching rates are extremely slow relative to total soil contamination
2. Biodegradation is much faster, allowing much faster site clean-up times

3. Soil contamination levels are difficult to determine with out a rigorous sampling regime

Section 4 Tables

Table 4.1

Flow Rate (ml/min)	Time of Conditions (day started - day ended)		
	Column One	Column Two	Column Three
0.13	305-333	222-262	220-260
0.3	335-368	264-285	262-283
0.51	400-427	317-344	316-342

Table 4.2

Column One						
PAH	Average Effluent Concentration (mg/L), Removal Rate mg/24h at:					
	Low Flow	Removal Rate	Med Flow	Removal Rate	High Flow	Removal Rate
Naphthalene	0.1073	0.0201	0.0768	0.0332	0.0422	0.0310
Acenaphthene	0.1440	0.0270	0.1185	0.0512	0.0830	0.0610
Fluorene	0.0787	0.0147	0.0600	0.0259	0.0434	0.0319
Phenanthrene	0.0797	0.0149	0.0542	0.0234	0.0465	0.0341
Column Two						
PAH	Average Effluent Concentration (mg/L), Removal Rate mg/24h at:					
	Low Flow	Removal Rate	Med Flow	Removal Rate	High Flow	Removal Rate
Naphthalene	0.0186	0.0035	0.0127	0.0055	0.0103	0.0076
Acenaphthene	0.0709	0.0133	0.0521	0.0225	0.0447	0.0328
Fluorene	0.0598	0.0112	0.0403	0.0174	0.0317	0.0233
Phenanthrene	0.0514	0.0096	0.0353	0.0152	0.0258	0.0189
Column Three						
PAH	Average Effluent Concentration (mg/L), Removal Rate mg/24h at:					
	Low Flow	Removal Rate	Med Flow	Removal Rate	High Flow	Removal Rate
Naphthalene	0.4165	0.0780	0.3606	0.1558	0.2750	0.1188
Acenaphthene	0.1118	0.0209	0.1061	0.0458	0.0788	0.0340
Fluorene	0.0815	0.0153	0.0731	0.0316	0.0565	0.0244
Phenanthrene	0.0617	0.0116	0.0655	0.0283	0.0530	0.0229

Table 4.3

Flow Rate (ml/min)	Time of Conditions (day started - day ended)		
	Column One	Column Two	Column Three
0.28	450-476	367-393	365-391
0.58	477-513	394-430	392-428

Table 4.4

Initial Soil Analysis - Column One		
Compound	mg/Kg Soil	Total Weight (mg)
Naphthalene	43.6	104.6
Acenaphthene	80.6	193.4
Fluorene	75.8	181.9
Phenanthrene	213.7	512.9
Initial Soil Analysis - Column Two		
Compound	mg/Kg Soil	Total Weight (mg)
Naphthalene	6.0	14.4
Acenaphthene	30.5	73.2
Fluorene	37.1	89.0
Phenanthrene	110.5	265.2
Initial Soil Analysis - Column Three		
Compound	mg/Kg Soil	Total Weight (mg)
Naphthalene	7.2	20.2
Acenaphthene	61.6	172.5
Fluorene	54.6	152.9
Phenanthrene	201.8	565.0

Table 4.5

Intermediate Port Soil Analysis - Column One					
Depth (in from Top)	Compound (mg/Kg)				
	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	foc
3	30.2	232.6	206.5	622.7	1.74
6	23.6	160.4	161.5	458.3	8.56
9	0	0	0	3.1	0.364
Intermediate Port Soil Analysis - Column Two					
Depth (in from Top)	Compound (mg/Kg)				
	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	foc
3	1	47.97	46.16	133.13	1.56
6	0	2.3	0.9	10.8	1.91
9	17.1	164.7	128.6	402.42	3.24
Intermediate Port Soil Analysis - Column Three					
Depth (in from Top)	Compound (mg/Kg)				
	Naphthalene	Acenaphthene	Fluorene	Phenanthrene	foc
3	0.5	3.6	7.4	42.5	1.58
6	161.6	358.4	371.1	1097.1	1.39
9	18.4	23.9	28.8	97.8	1.15

Table 4.6

Column One					
Compound	Total Weight After Treatment (mg)	Leached Amount (mg)	Amount Biodegraded (mg)	Percent Removed	Total Weight before Treatment (mg)
Naphthalene	6.6	30.5			104.6
Acenaphthene	66.6	18.3			193.4
Fluorene	55.3	16.6			181.9
Phenanthrene	232.2	11.3			512.9
Totals	360.7	76.7	364.0	55.0	992.8
Mass Balance Error (%) 19.3					
Column Two					
Compound	Total Weight After Treatment (mg)	Leached Amount (mg)	Amount Biodegraded (mg)	Percent Removed	Total Weight before Treatment (mg)
Naphthalene	7.9	6.5			14.4
Acenaphthene	97.6	32.7			73.2
Fluorene	75.0	24.6			89.0
Phenanthrene	291.0	21.7			265.2
Totals	471.5	85.5	310.0	45.6	441.8
Mass Balance Error (%) 96.2					
Column Three					
Compound	Total Weight After Treatment (mg)	Leached Amount (mg)	Amount Biodegraded (mg)	Percent Removed	Total Weight before Treatment (mg)
Naphthalene	104.1	71.4			20.2
Acenaphthene	250.2	15.4			172.5
Fluorene	203.2	12.3			152.9
Phenanthrene	579.8	10.5			565.0
Totals	1137.3	109.6	310.0	27.0	910.6
Mass Balance Error (%) 71.0					

Section 4 Figures

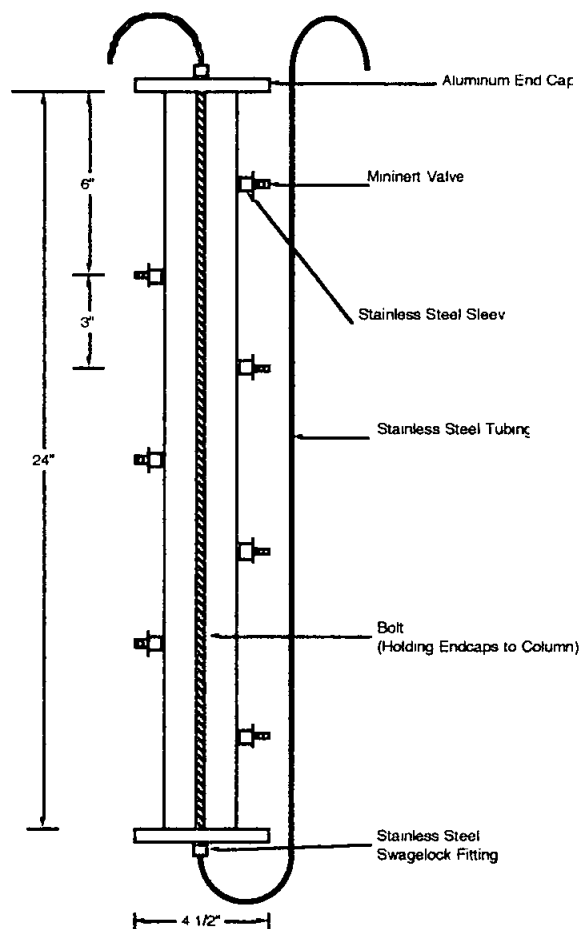


Figure 4.1 Schematic of a 2.5-inch Diameter Stainless Steel Column

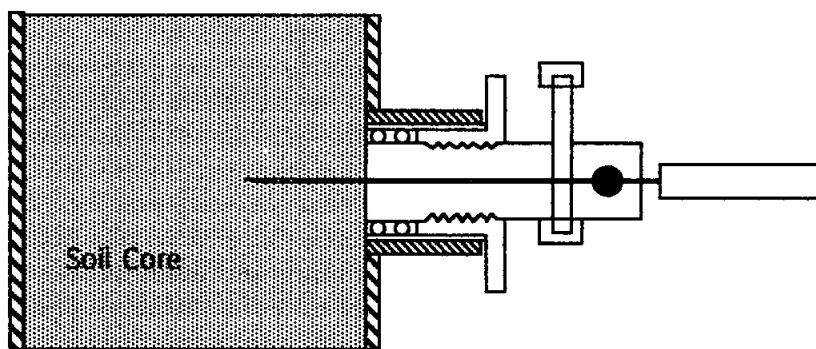


Figure 4.2 Cut-Away View of a Mininert Valve

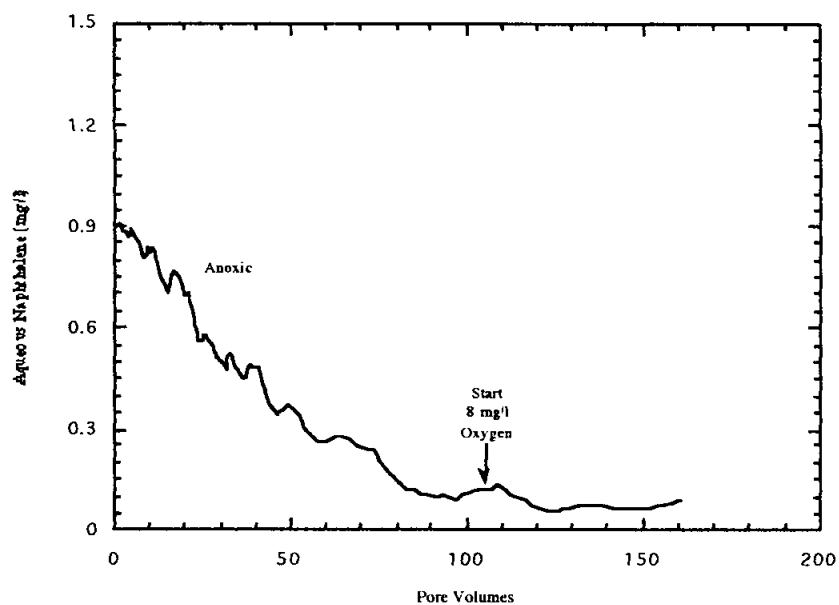


Figure 4.3 Column One: Naphthalene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

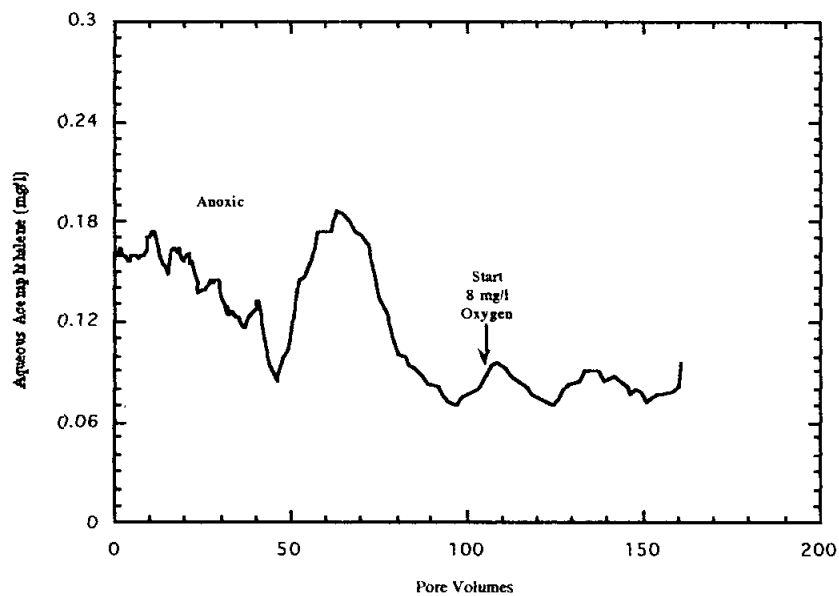


Figure 4.4 Column One: Acenaphthene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

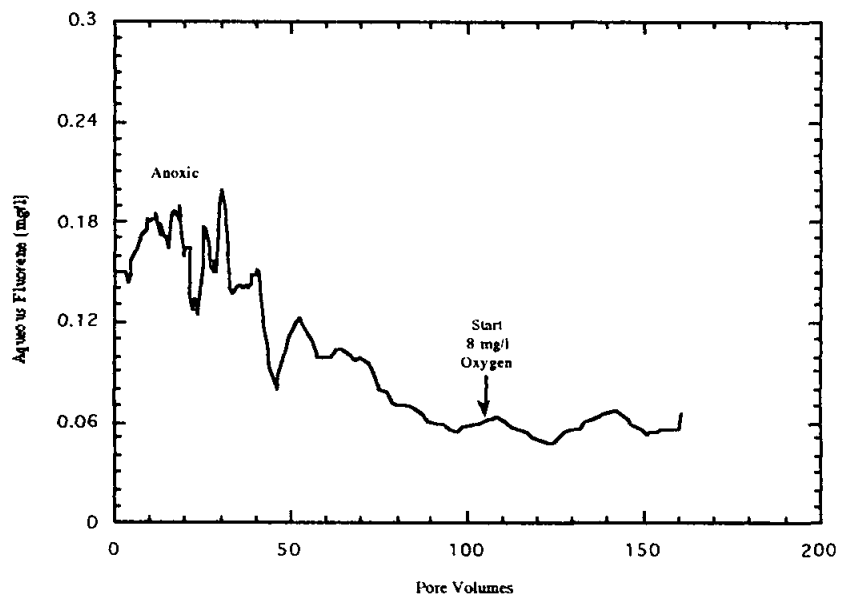


Figure 4.5 Column One: Fluorene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

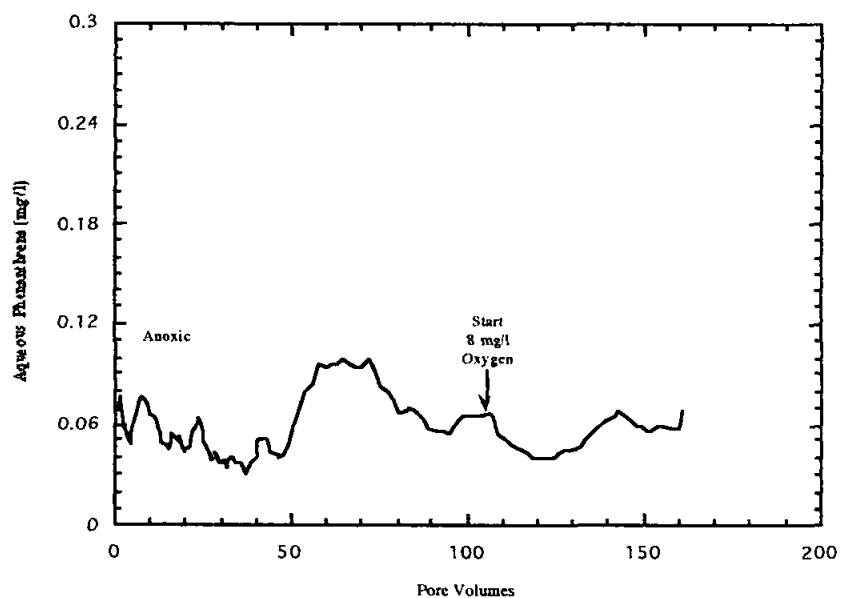


Figure 4.6 Column One: Phenanthrene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

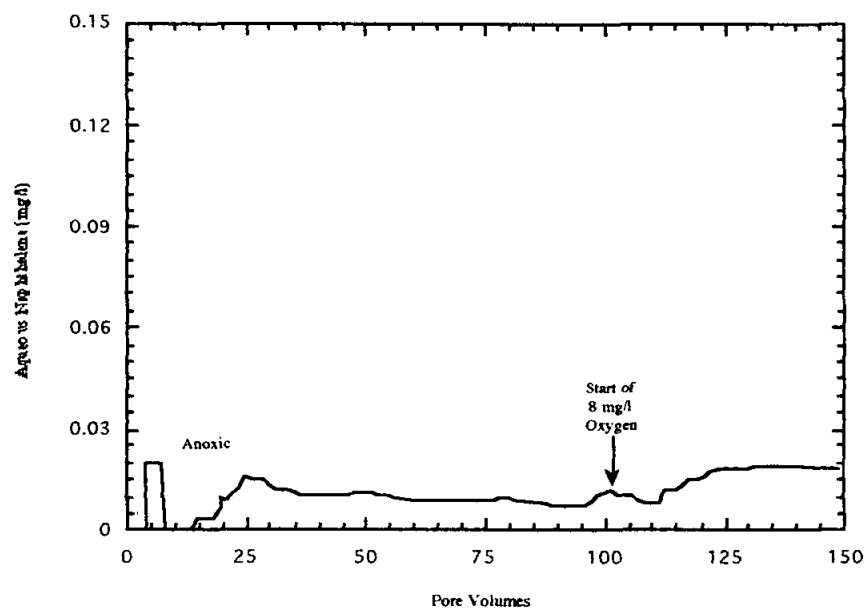


Figure 4.7 Column Two: Naphthalene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

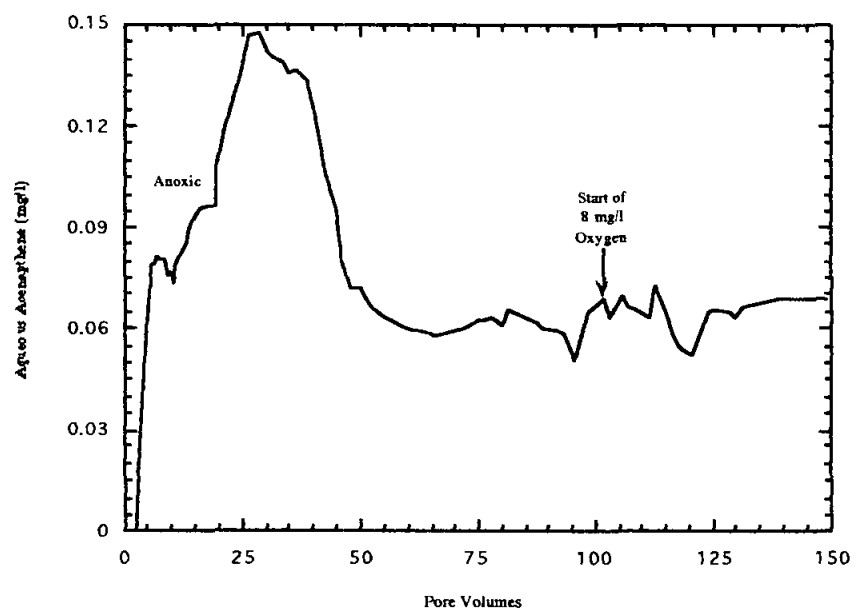


Figure 4.8 Column Two: Acenaphthene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

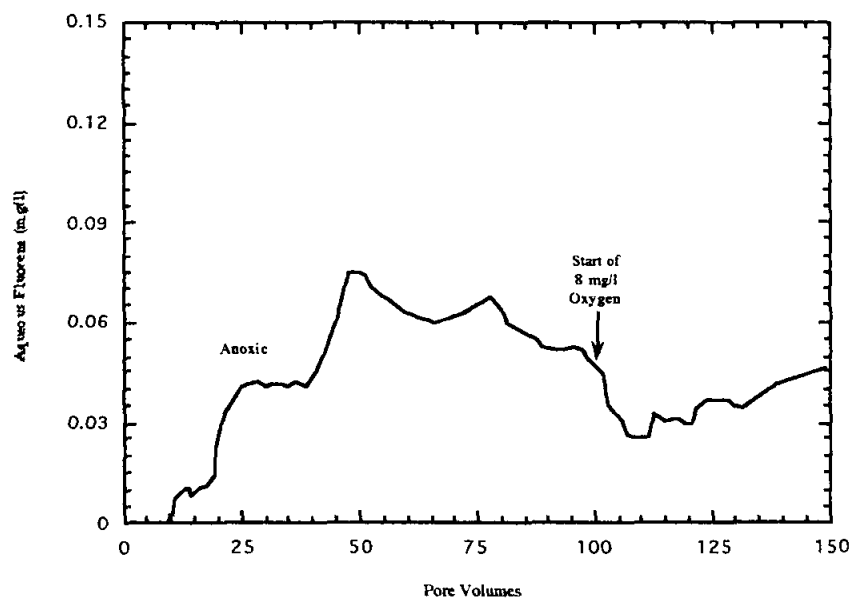


Figure 4.9 Column Two: Fluorene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

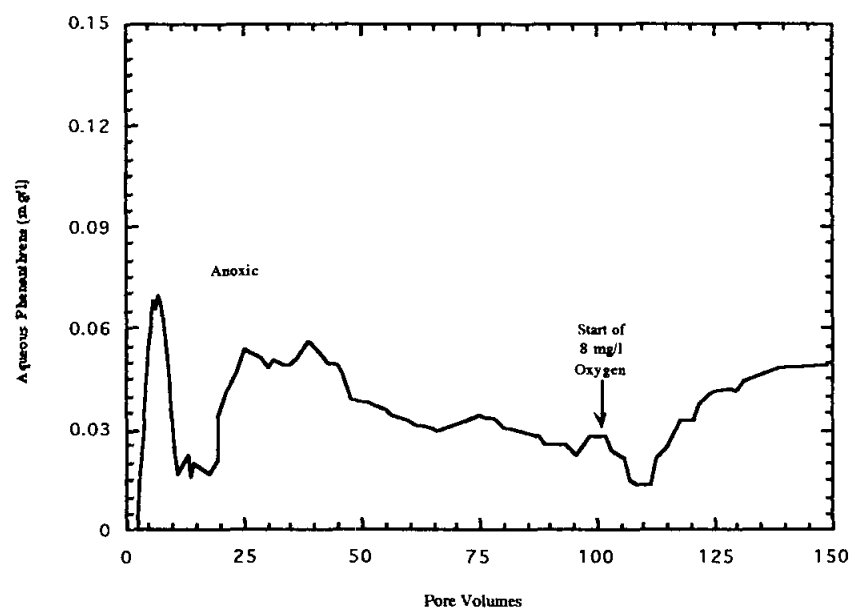


Figure 4.10 Column Two: Phenanthrene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

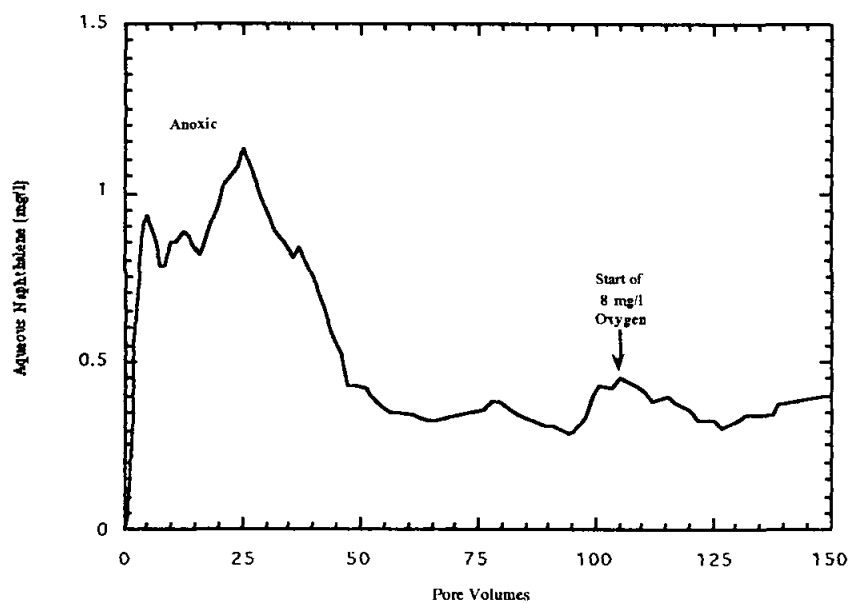


Figure 4.11 Column Three: Naphthalene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

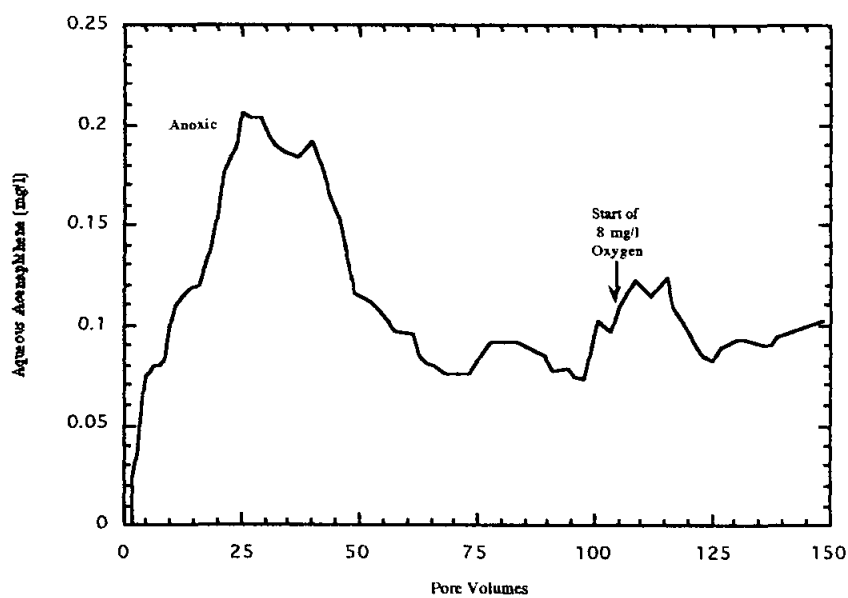


Figure 4.12 Column Three: Acenaphthene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

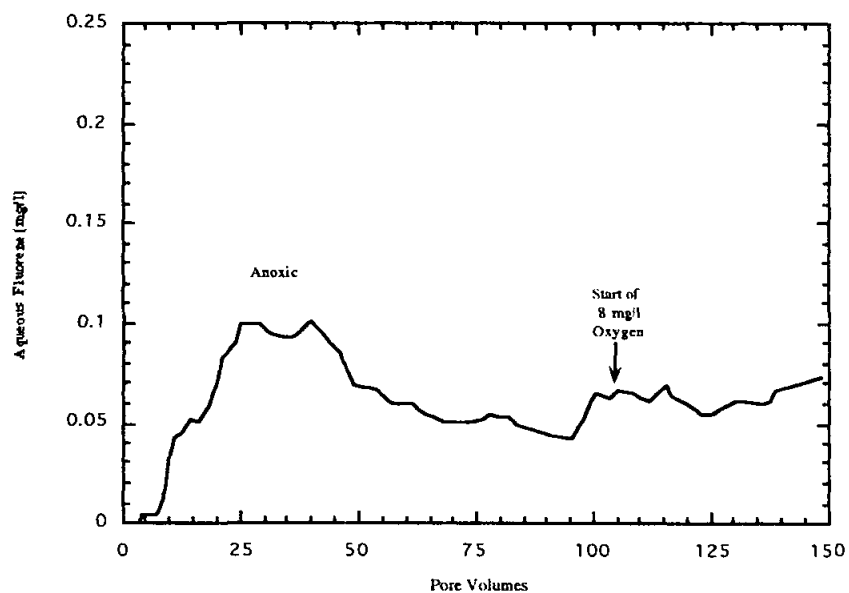


Figure 4.13 Column Three: Fluorene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

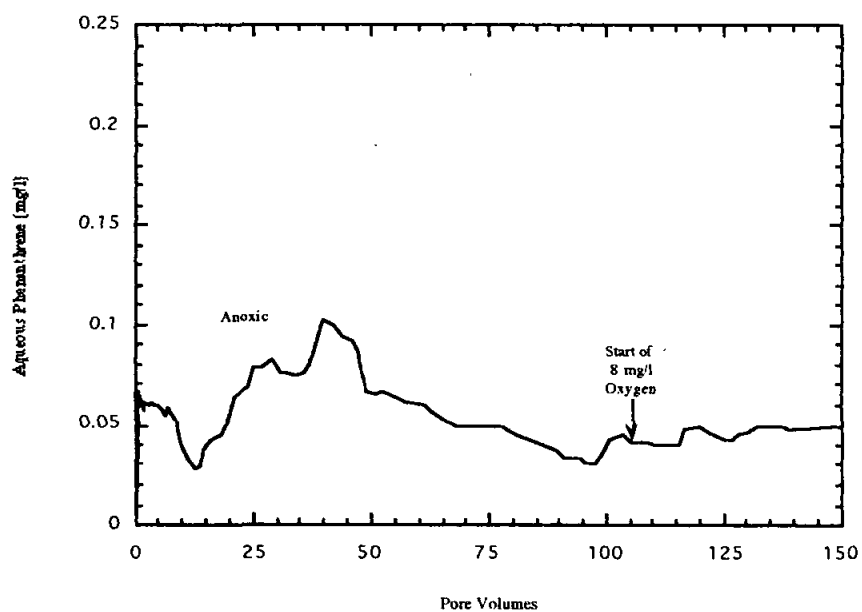


Figure 4.14 Column Three: Phenanthrene Effluent Concentration Under Anoxic and Ambient Oxygen Conditions

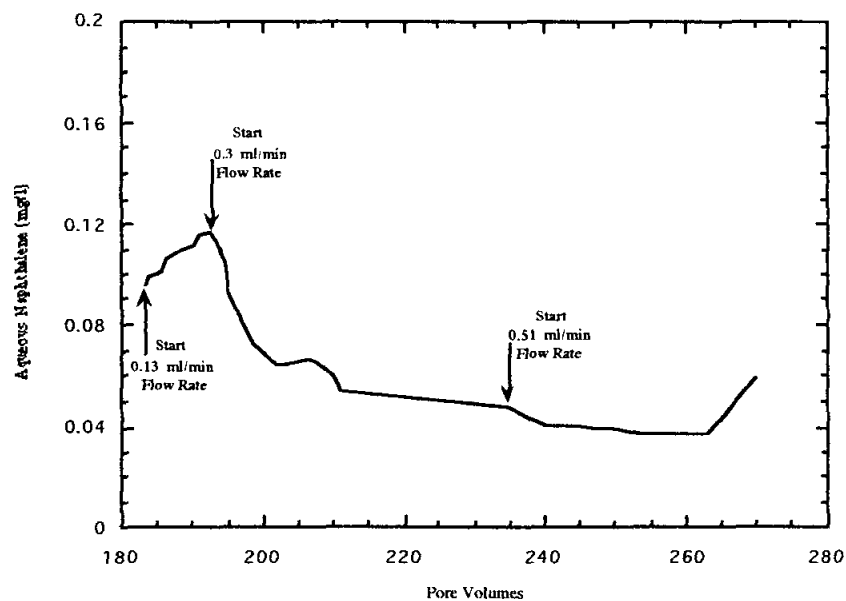


Figure 4.15 Column One: Naphthalene Effluent Concentration Under Varying Flow Rate Conditions

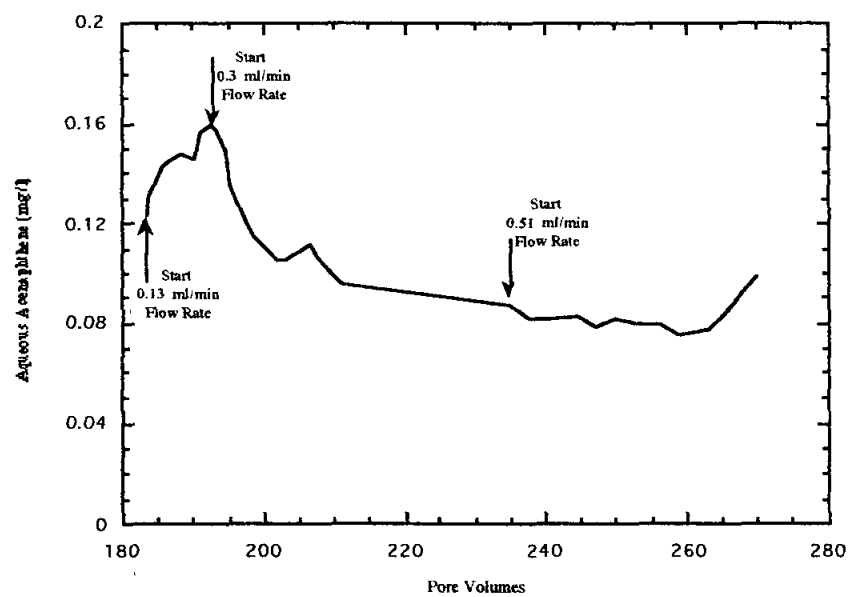


Figure 4.16 Column One: Acenaphthene Effluent Concentration Under Varying Flow Rate Conditions

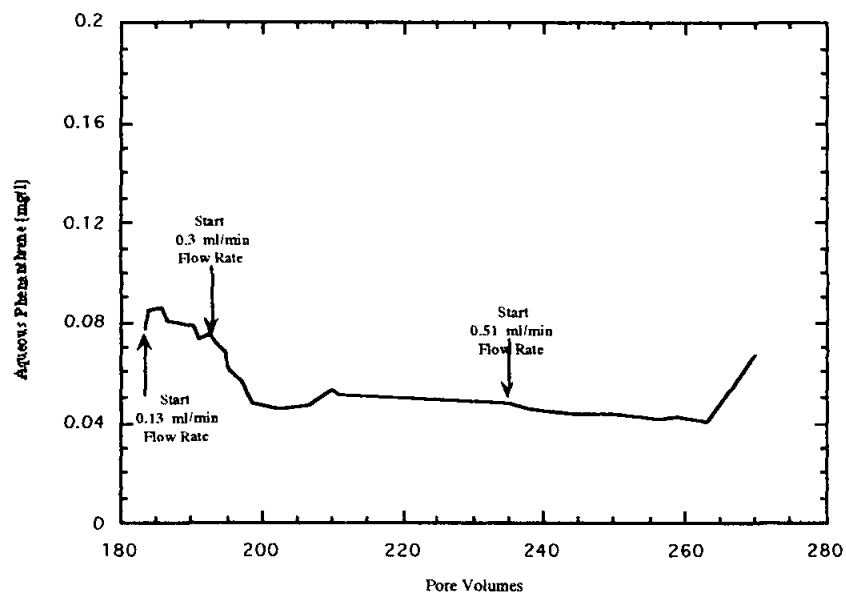


Figure 4.17 Column One: Phenanthrene Effluent Concentration Under Varying Flow Rate Conditions

Figure 4.18 Column One: Fluorene Effluent Concentration Under Varying Flow Rate Conditions

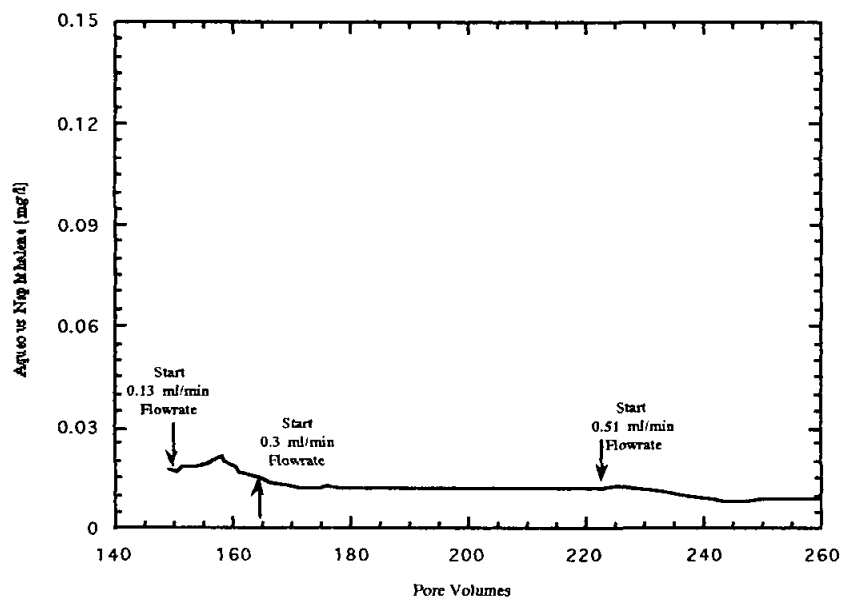


Figure 4.19 Column Two: Naphthalene Effluent Concentration Under Varying Flow Rate Conditions

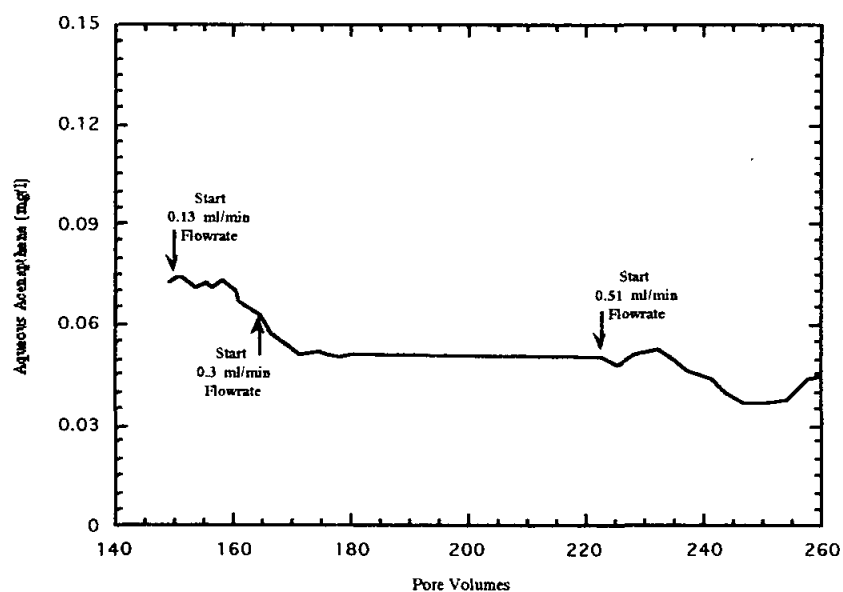


Figure 4.20 Column Two: Acenaphthene Effluent Concentration Under Varying Flow Rate Conditions

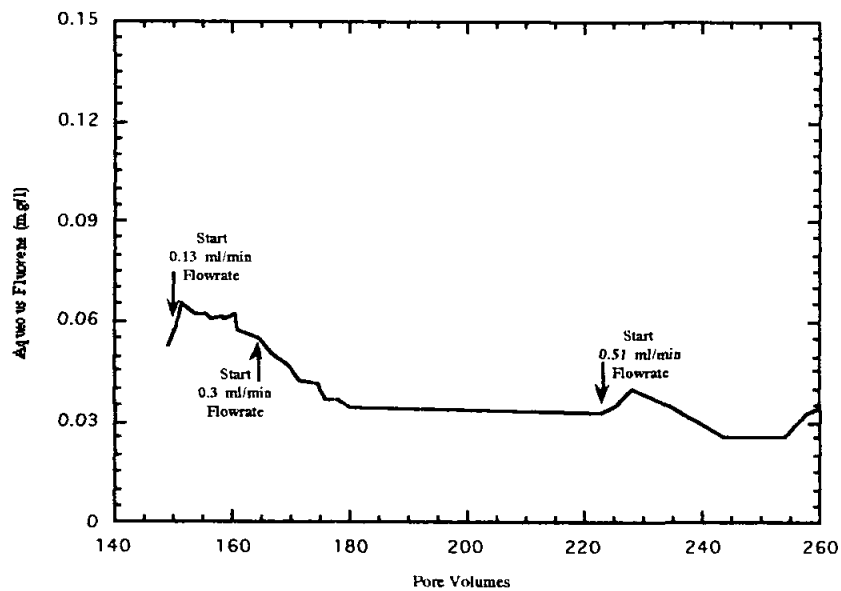


Figure 4.21 Column Two: Fluorene Effluent Concentration Under Varying Flow Rate Conditions

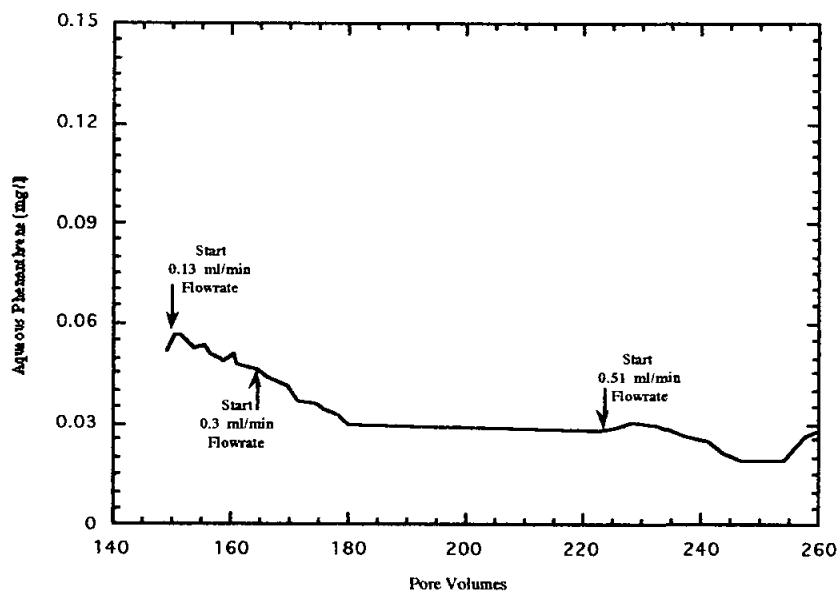


Figure 4.22 Column Two: Phenanthrene Effluent Concentration Under Varying Flow Rate Conditions

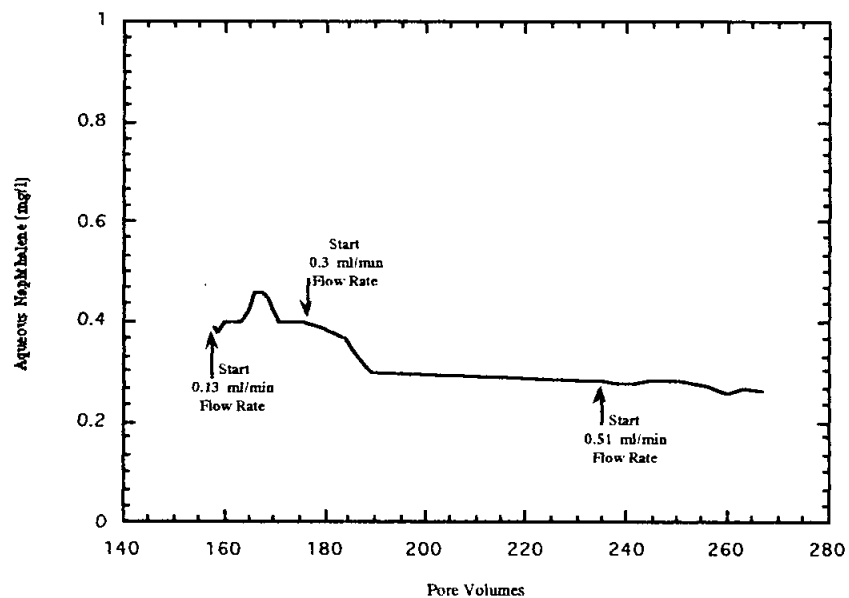


Figure 4.23 Column Three: Naphthalene Effluent Concentration Under Varying Flow Rate Conditions

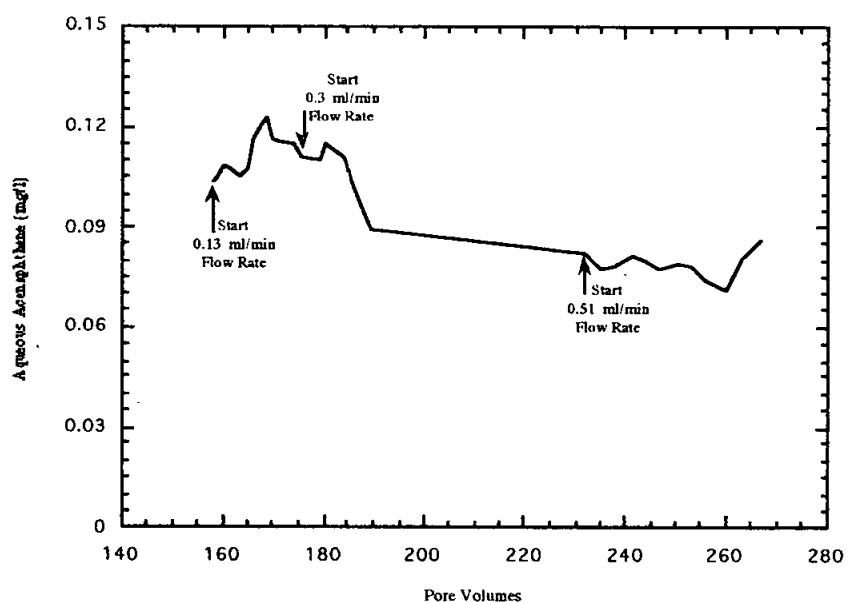


Figure 4.24 Column Three: Acenaphthene Effluent Concentration Under Varying Flow Rate Conditions

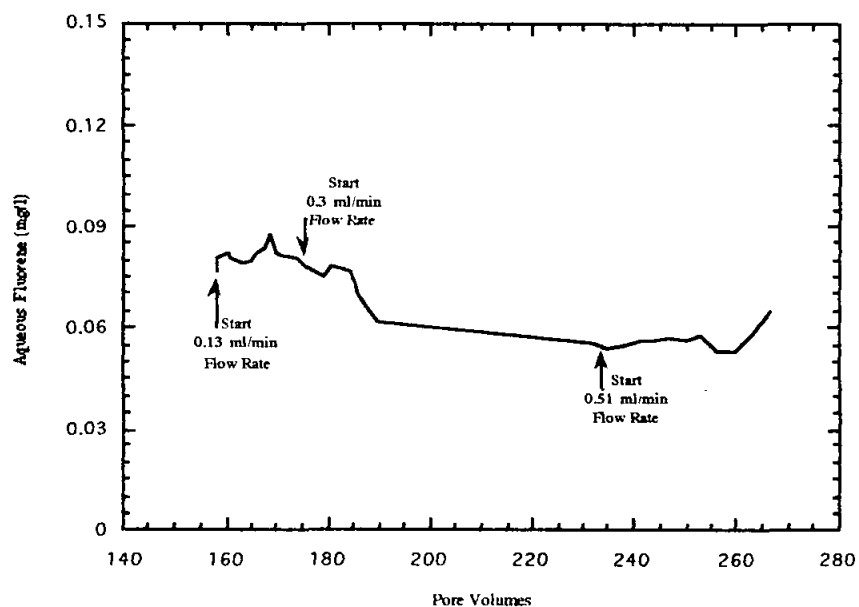


Figure 4.25 Column Three: Fluorene Effluent Concentration Under Varying Flow Rate Conditions

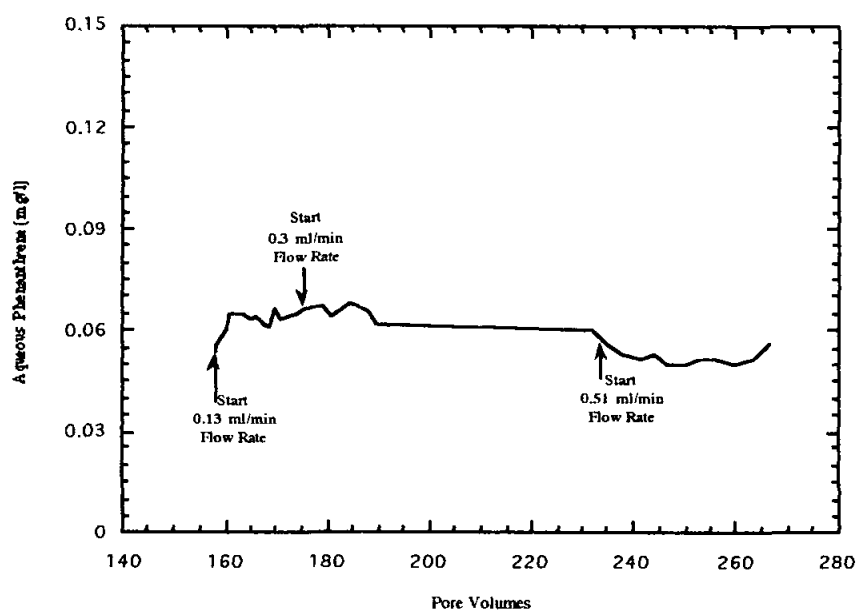


Figure 4.26 Column Three: Phenanthrene Effluent Concentration Under Varying Flow Rate Conditions

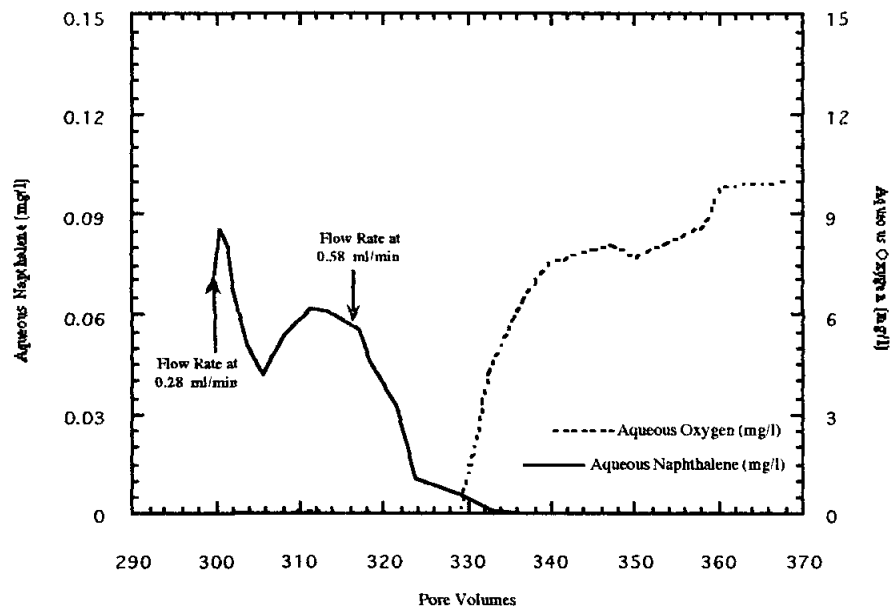


Figure 4.27 Column One: Naphthalene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

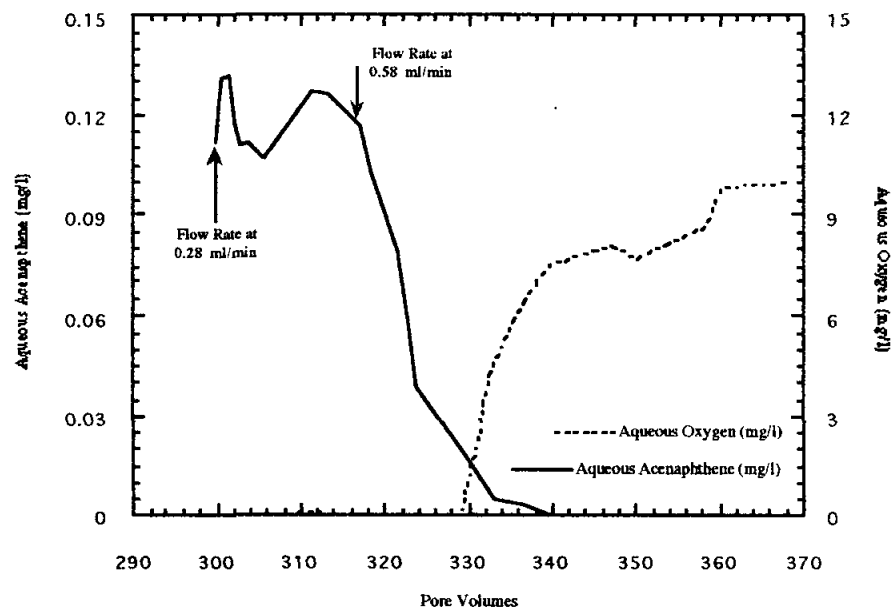


Figure 4.28 Column One: Acenaphthene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

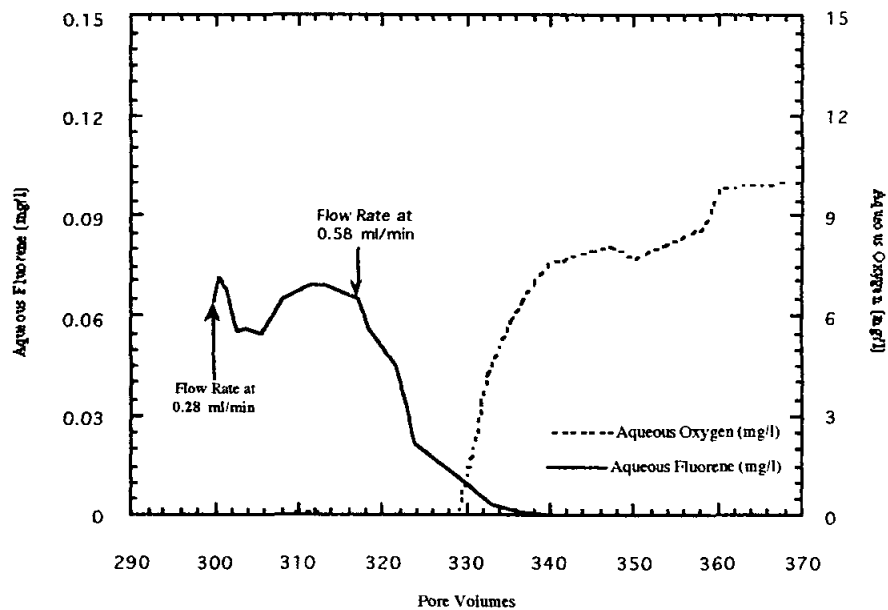


Figure 4.29 Column One: Fluorene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

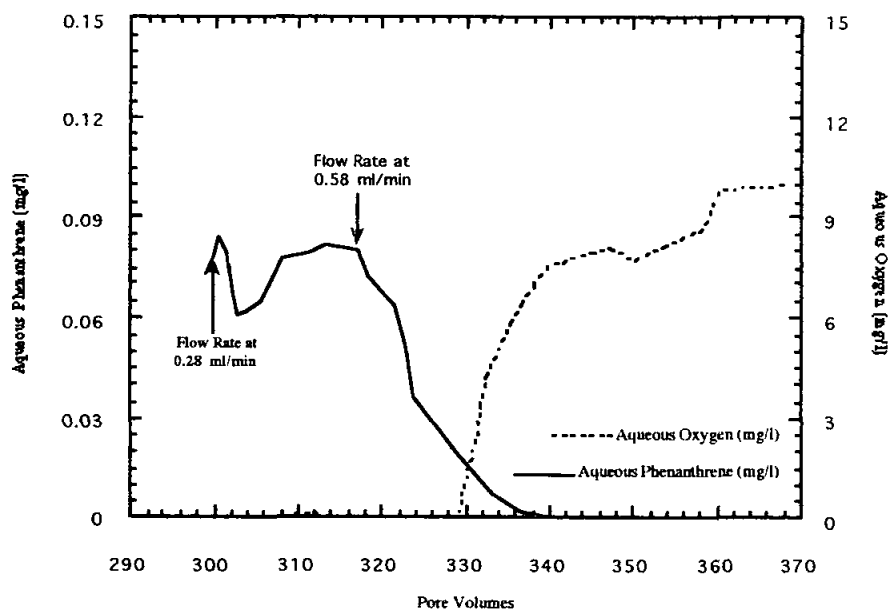


Figure 4.30 Column One: Phenanthrene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

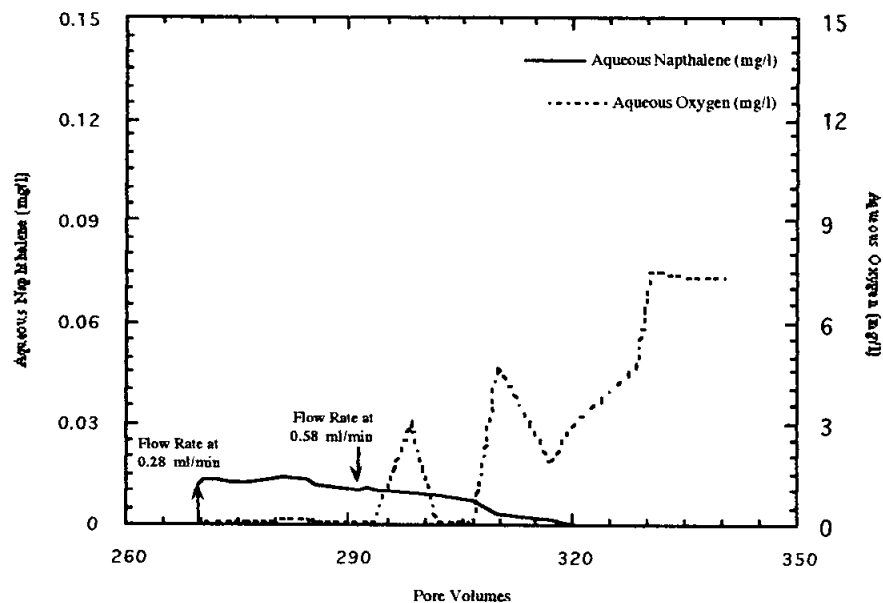


Figure 4.31 Column Two: Naphthalene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

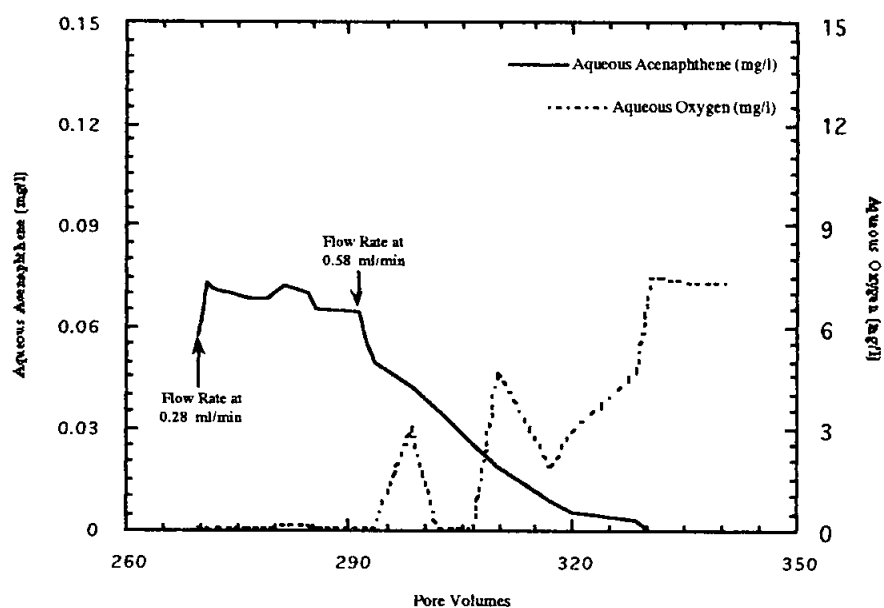


Figure 4.32 Column Two: Acenaphthene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

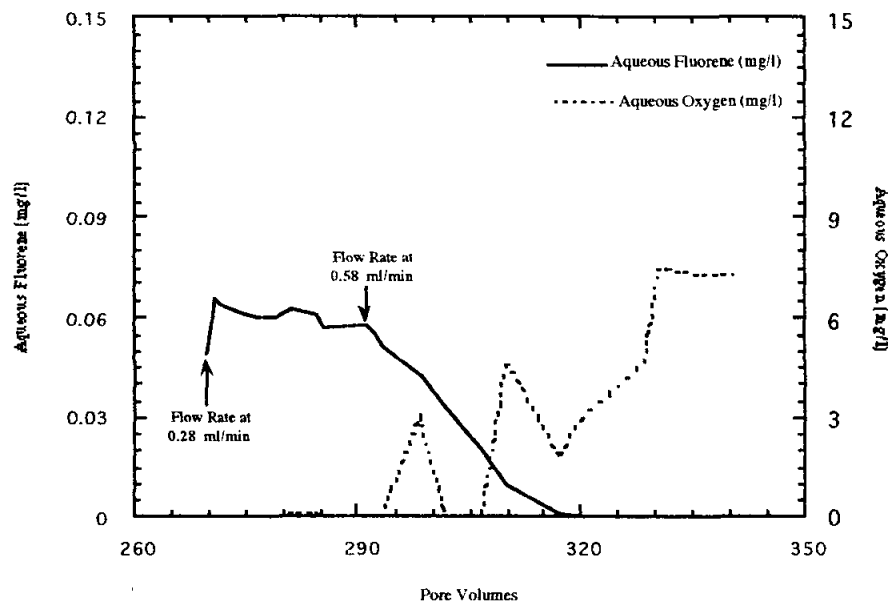


Figure 4.33 Column Two: Fluorene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

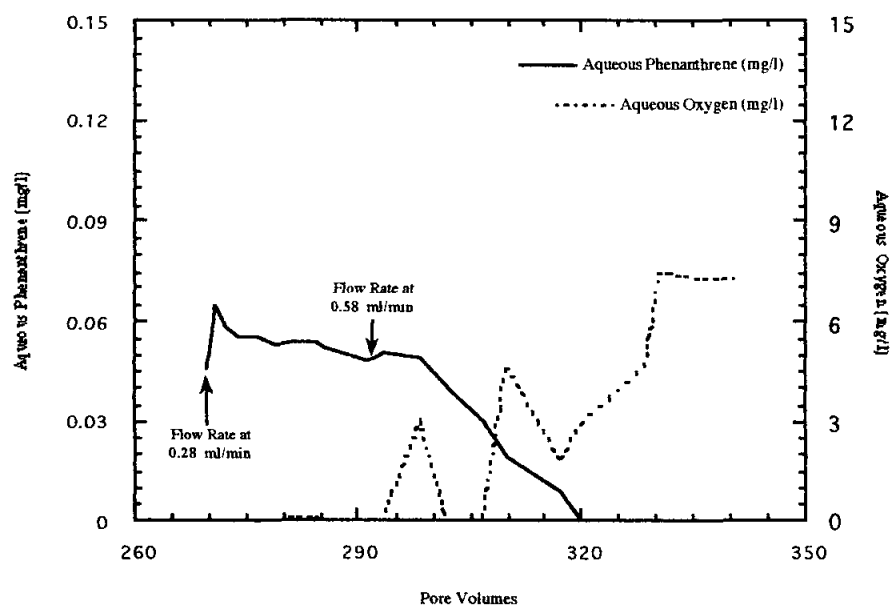


Figure 4.34 Column Two: Phenanthrene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

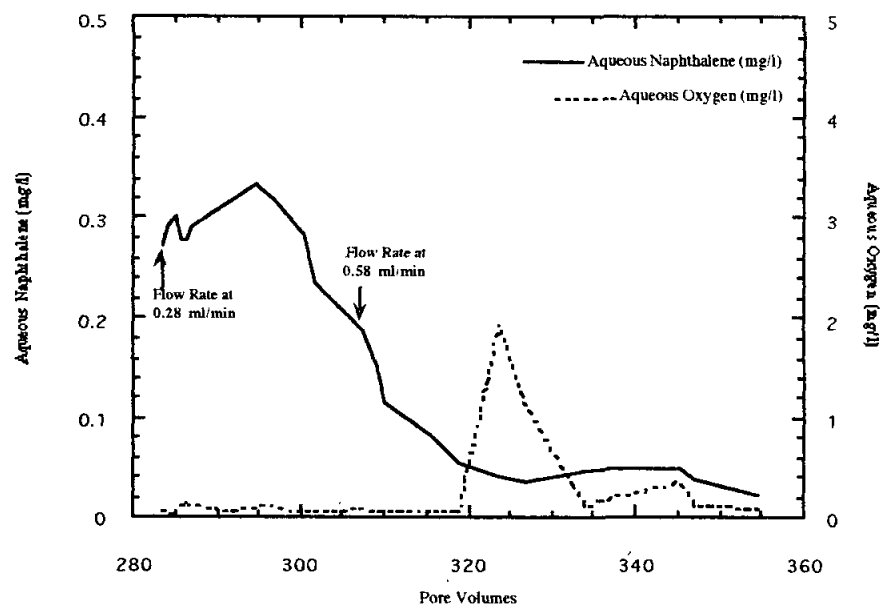


Figure 4.35 Column Three: Naphthalene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

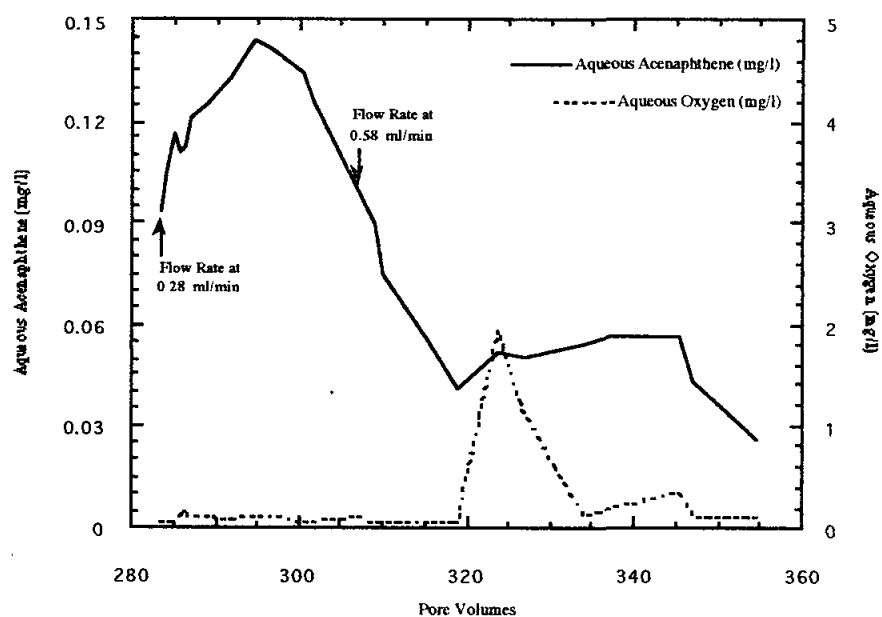


Figure 4.36 Column Three: Acenaphthene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

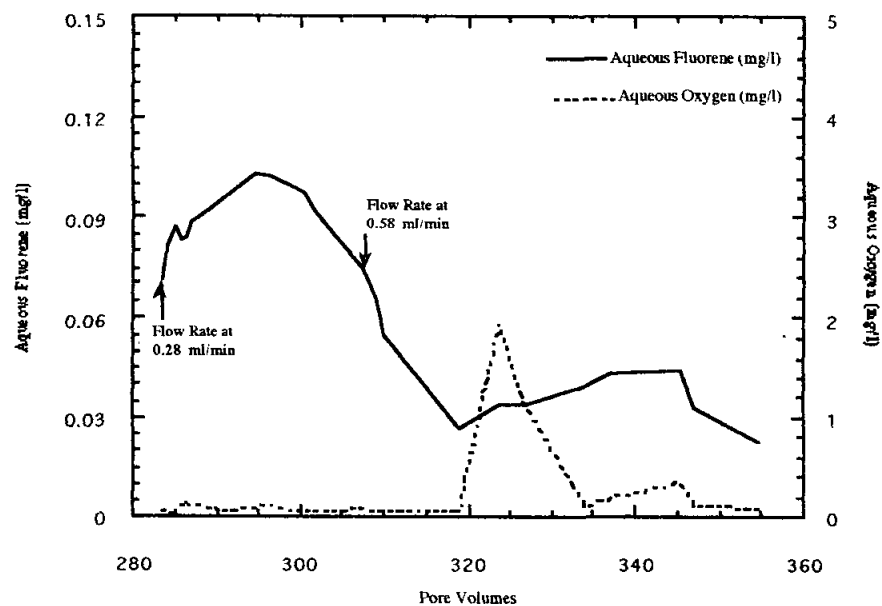


Figure 4.37 Column Three: Fluorene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

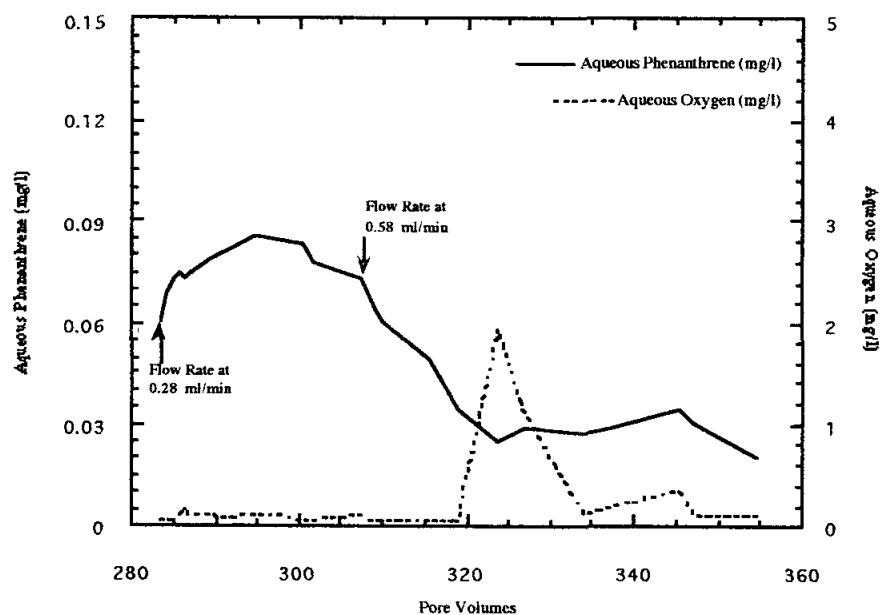


Figure 4.38 Column Three: Phenanthrene and Oxygen Effluent Concentrations Under Saturated Oxygen Conditions with Varying Flow Rates

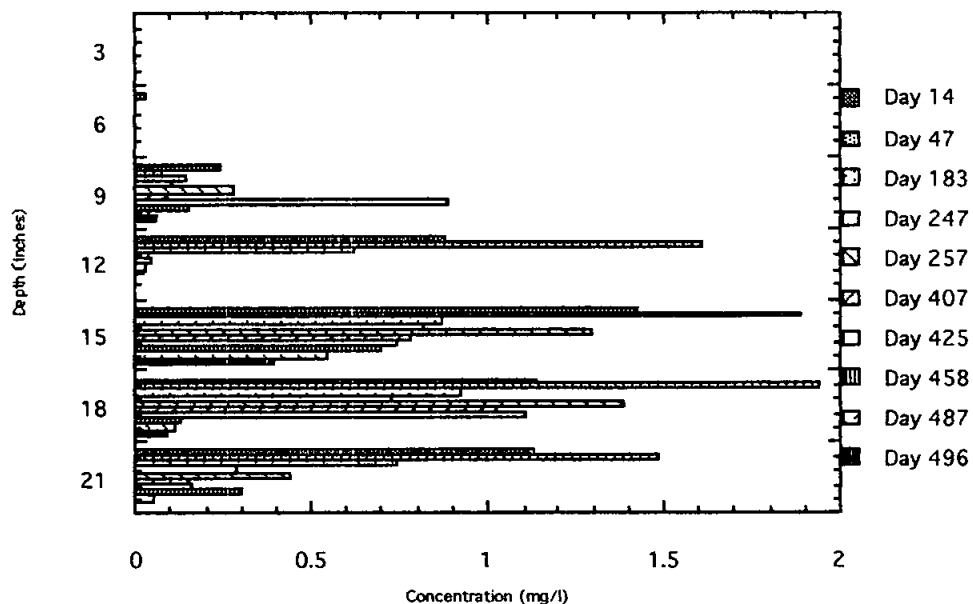


Figure 4.39 Total Aqueous PAH Profiles from Column One Sampling Ports

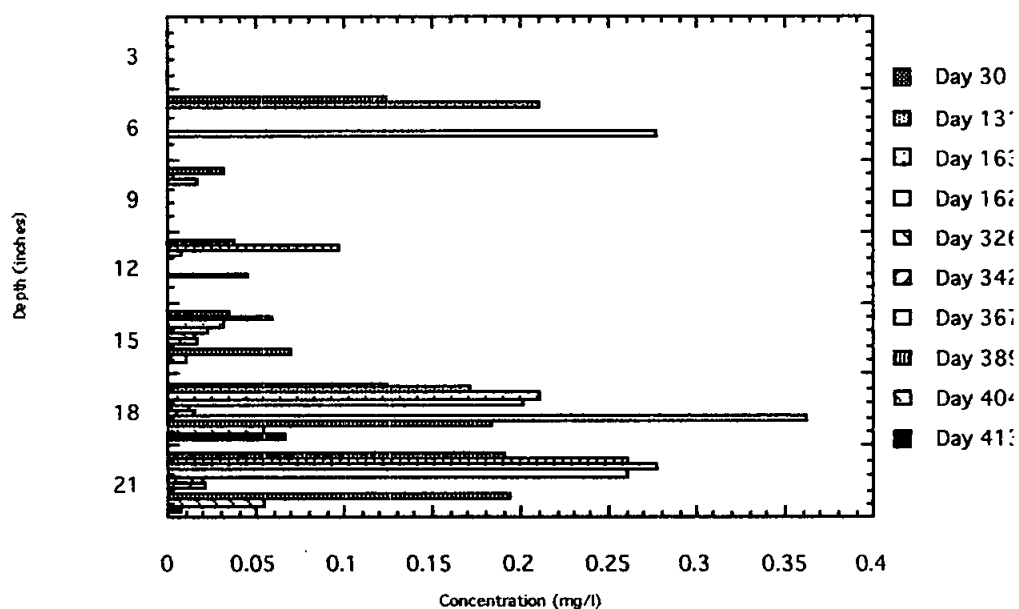


Figure 4.40 Total Aqueous PAH Profiles from Column Two Sampling Ports

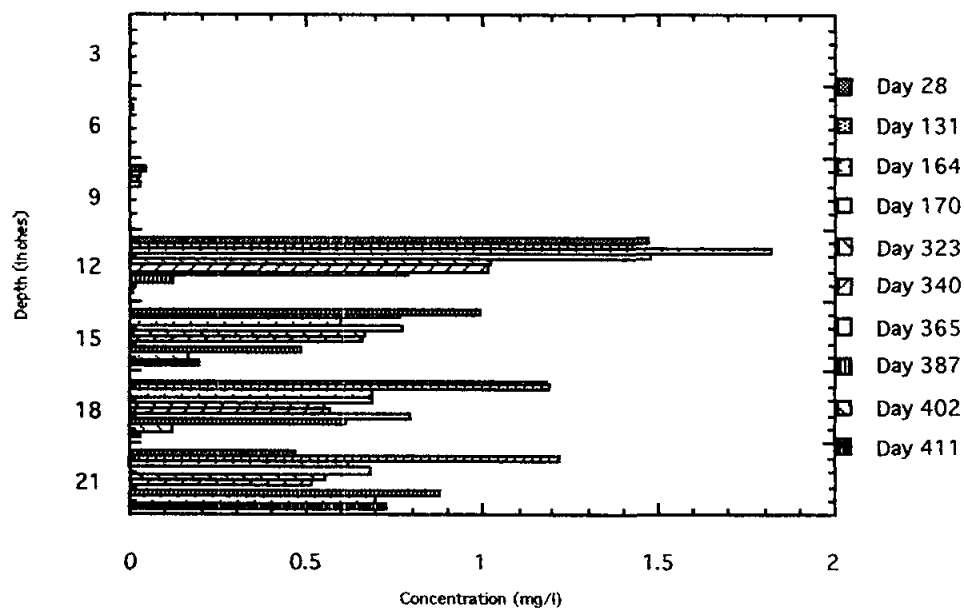
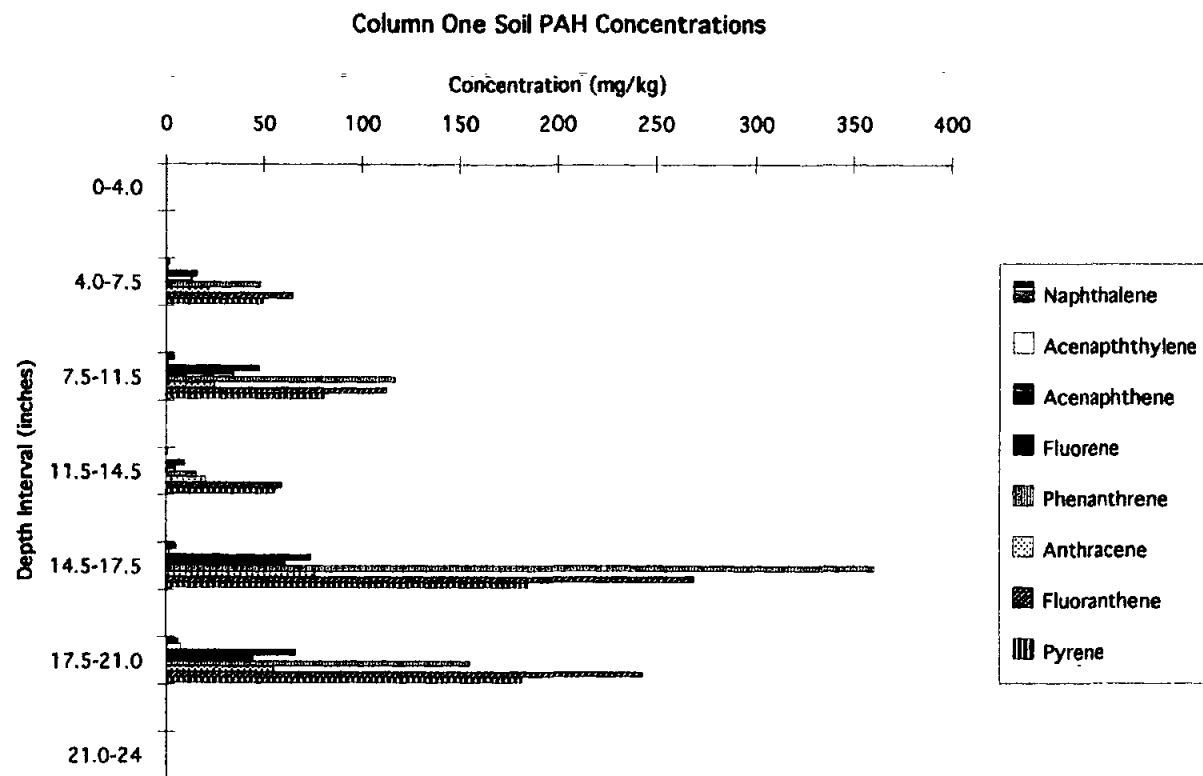


Figure 4.41 Total Aqueous PAH Profiles from Column Three Sampling Ports

135



136

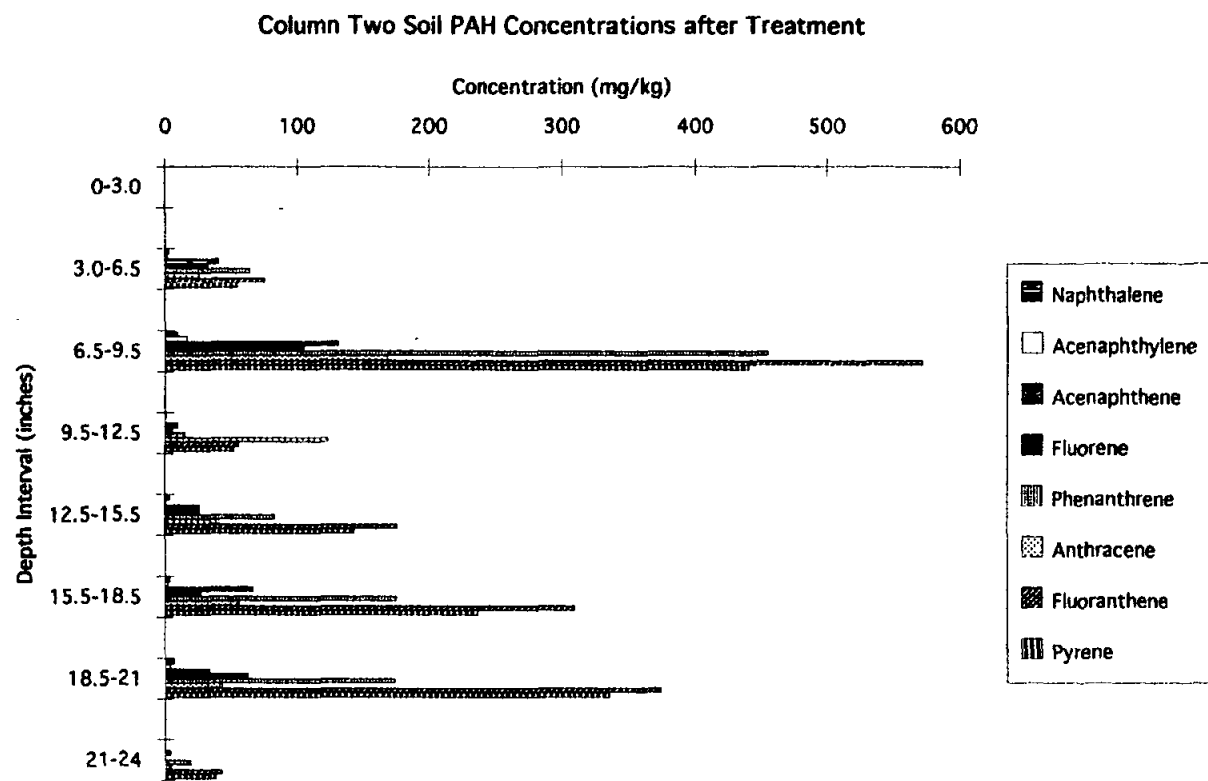


Figure 4.44 Column Three Soil PAH Concentrations After Treatment

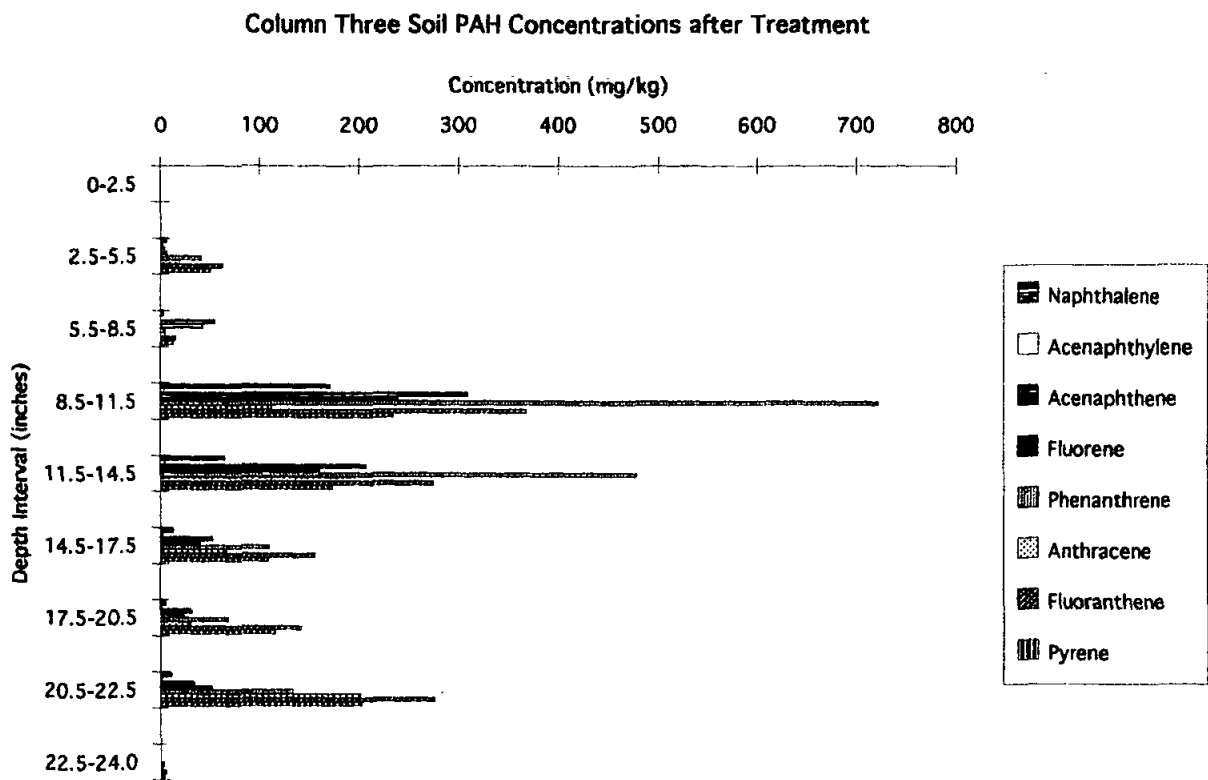
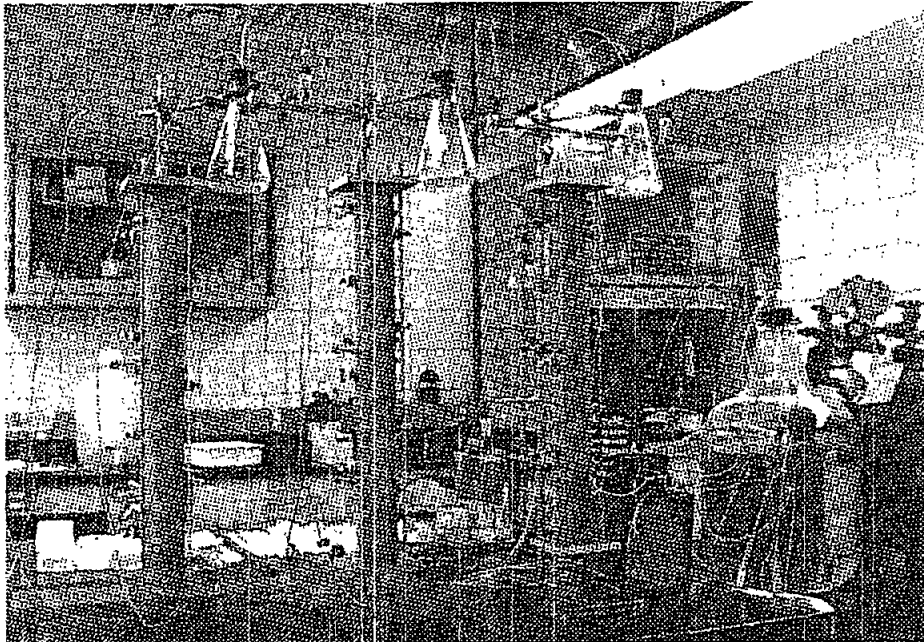


Plate 4.1 Setup of the Split Spoon Insert Columns Study



Section 5

Modeling Studies

Abbreviated Report on Modelling Transport in Porous Media with Biodegradation and Solubilization of Excess Substrate (TPMBX2)

The TPMBX2 program was developed as part of the Reilly study to facilitate interpretation of the data were generated in the laboratory column studies described in previous sections. The available computer programs were found inadequate for several reasons, namely:

1. they do not explicitly incorporate the effects of oxygen limitations on the kinetics of biodegradation
2. they do not account for time and spatial changes in biomass concentration distributions as a function of time resulting from growth and endogenous decay processes
3. they do not explicitly describe changes in time and spatial concentration distributions of pollutants
4. most of the simpler models ascribe the retention of pollutants to adsorption rather than separate phase occlusions of the pollutant chemicals
5. a related deficiency is that most of the simpler models do not consider solubilization of the separate phase chemicals as a potentially rate limiting process.

This part of the report describes the salient features of TPMBX2 namely, a) documentation of the transport equations b) descriptions of the rate equations used to describe the kinetics of solubilization and biodegradation, c) applications of the model for analyzing column data. This abbreviated report presents those parts of the report that are likely to be of greatest interest to people who are interested in applications. The complete report which includes the topics shown on the table of contents (next page) will be made available separately.

**Abbreviated Report on
Documentation, Application, and Evaluation of the TPMBX2
Program**

Contents:

1. Model information and system equations
 - a. Advective/Dispersive equation for soluble substrate transport
 - b. Advective/Dispersive equation for oxygen transport
 - c. Haldane-type model of bacterial growth
 - d. Mass transfer equation of separate phase chemical dissolving into water phase
 - e. Boundary conditions for flow in a finite soil column
2. A User's Guide to TPMBX2
 - a. Hardware requirements
 - b. Governing equations with boundary conditions
 - c. Runge-Kutta fourth-order ODE solution method
 - d. Operating TPMBX2
 1. Getting Started
 2. Selection menu
 3. Editing (data sets, constants, and units)
 4. The data sets menu
 5. A note on physical units
 6. Computational mode - how to interpret the Runge-Kutta Solver
 7. Data Output - Plotting data
 8. Storing/Retrieving data - the Input/Output menu and other means
3. Sensitivity analysis and default values for required parameters
 - a. Sensitivity analysis of required parameters
 - b. Default values for required parameters (aerobic degradation only)
4. Applications of TPMBX2
 - a. Application to a Base Case example - Flushing a finite column of contaminated soil with oxygenated water
 - b. Application to the Reilly Site treatability study
 1. Adjustment of parameters to account for observed mass transfer difficulties
 2. Short-term model predictions
 3. Long-term model predictions
 - c. Application to another Base Case example - Treating a large-scale spill with in-situ biodegradation
 1. Modelling growth and extent of untreated plume (natural degradation only)
 2. Modelling use of single injection well to treat plume
 3. Modelling use of regularly spaced injection wells to treat plume
5. Suggested model modifications

1. Model information and system equations

TPMBX2 is a computational model that simulates mass transport of a soluble substrate through a finite oxygenated soil column while undergoing aerobic biodegradation and liquid-liquid mass transfer from a separate, excess phase of chemical. Thus, the PC program TPMBX2 models Transport in Porous Media with Biodegradation and solubilization of eXcess substrate. (It's the second version of the program. Hence the acronym.)

The output is an approximation of time-space concentration distributions of soluble organic chemical under saturated [groundwater] flow conditions in which oxygen and biomass concentrations are also time-space variable. The concentration of soluble chemical available for biodegradation is modelled by a time-dependent mass transfer of separate phase organic chemical into the aqueous phase (where biodegradation occurs). The system can then be described by a series of four coupled partial differential equations modelling the subsurface transport of both substrate and oxygen, the dissolution rate of excess substrate within the soil column, and the accumulation of active biomass due to substrate digestion and oxygen utilization.

Thus advection, dispersion, retardation, biodegradation, and solubilization of both the organic chemical and (when pertinent) oxygen are described by that series of equations. TPMBX2 uses a fourth-order Runge-Kutta method to approximate the solutions to these equations and provides user-friendly graphical and numeric output.

The potential applications of this program include modelling the increasingly important practice of insitu biodegradation of organics in polluted aquifers. And, in the short term, TPMBX2 will be used to simulate a treatability study currently being carried out with minimally disturbed core samples taken from a Superfund site (the "Reilly Site") in St. Louis Park, Minnesota.

In the sections that follow equations will be developed that describe each of the four time dependent phenomena mentioned above. The transport equations used to model the movement of solute and oxygen through the column have been previously described in the literature and are fairly well understood. (R.S. Maier, 1991) A Haldane-type model will describe microbial growth on the substrate. (This is a Monod model modified to take into account competitive inhibition by excess substrate that reversibly binds the active biomass.) (Klecka and Maier, 1988) The Haldane model is also widely accepted in the literature as a valid description of microbial growth. (Although after Rittman and McCarty [1980] increased attention has been given growth kinetics modelled by stationary biofilms (or stationary colonies) in both surface and subsurface environments, a Haldane model is appropriate for this unidirectional, steady state, mostly advective flow.)

Finally, a model will be developed to describe the intraphase mass transfer of liquid solute into solution. This model was developed by Dr. Walter Maier and is used here to simulate the kinetics of phase transfer that limit the amount of substrate available for biodegradation at any time.

4. Applications of TPMBX2

The following case studies represent applications of TPMBX2 to actual or theoretical in-situ bioremediation problems. The first application is a theoretical scenario ("base case") involving the flushing of a finite contaminated soil column with clean, oxygenated water. TPMBX2 will model the flushing process, providing predictions of biomass growth, and oxygen and substrate depletion both in the short term (after one pore volume has been flushed), and in the long term (after 20 days of operation).

The second application models a treatability study currently being carried out on minimally disturbed core samples taken from a Superfund site (the "Reilly site") in St. Louis Park, Minnesota. This soil is heavily contaminated with creosote wastes deposited by the Reilly Tar Company over 60 years of railroad tie soaking operations. The company ceased operations in the mid-1970's, and the site is currently in the feasibility study phase of CERCLA mandated clean-up.

This application has many similarities to the "base case" but differs in the rate of mass transfer from the excess phase. Here, mass transfer for any one solute is hampered by the remaining solutes present in the heterogenous phase. (Many compounds have been identified in the soil including phenanthrene, fluoranthene, pyrene, anthracene, acenaphthene, fluorene, chrysene, and naphthalene. The total excess phase of pollution is estimated at 850 mg/kg dry soil.) Saturated column studies over 3 months of continuous operation have not produced saturated levels of any one of the several pollutants identified by gas chromatography. (In fact, even the most soluble of the substrates is present at only 10% of it's saturated value.) To account for this discrepancy in mass transfer, the excess phase is modelled as a near-cubical shaped phase (that limits the rate of transfer), but, more importantly, the C_s values are estimated at 10% of the theoretical values (to limit the

equilibrium values of substrate present for biodegradation). This combination of constants seems to satisfactorily model the kinetics of the system, but more adjustments will be required when kinetic parameters are determined later this summer (1992).

The third application involves the use of TPMBX2 to track the growth and extent of plume formation (in one dimension) following a small spill of highly degradable, moderately adsorbing wastes. The plume is tracked first in the presence of natural microbes without treatment, then with hollow fiber membrane treatment only, and lastly with the aid of an H_2O_2 solution injected from the surface via a driven well.

4a. Application to a "Base Case" Example - Flushing a Finite Column of Contaminated Soil with Oxygenated Water

The input values for the base case are those listed in section 4b. Figures 5 through 16 represent actual graphical output of TPMBX2 modelling this system. Figure 5 shows the concentrations of substrate (C), oxygen (CO), excess substrate (Cx), and biomass (Cb) throughout the column length after one pore volume has been flushed. (The residence time of the column is 0.82 days.) The equilibrium curve of excess substrate (a stationary phase) indicates dissolution to soluble substrate throughout the column to the theoretical value, C_s . The soluble substrate near the inlet has undergone some (very little) biological degradation and is beginning to rapidly advect through the column ($R_c = 10$). Only a small oxygen demand has developed as biomass concentrations start out initially very low ($C_{bi} = 0.200$ mg/l).

After 5 days of flushing (figure 6), excess substrate has been significantly removed through the first part of the column. Biomass growth has by now become more significant, with a majority of growth in the top half of the column. As a result of the

aggressively growing biomass, an oxygen deficit has occurred. The column is operating anaerobically after about 30 cm. (This slows microbial growth, allowing substrate to freely advect through the bottom half of the column.)

Figure 7 shows the on-screen output of TPMBX2 corresponding to figure 6 (i.e. after 5 days of throughput). The self-scaled axes allow for a clearer view of changes in each variable. Figure 8 shows similar output after 7.6 days of growth. Again a tremendous increase in biomass (and a shift in the distribution of biomass in the column) indicates aggressive substrate digestion. At this point, the excess substrate is nearly depleted, a significant portion has been digested, while some has passed through the outlet as soluble substrate. [The presence of near equilibrium values of soluble substrate at the end of the column is not surprising given the oxygen deficit there.]

Figure 9 shows the concentration/depth profile in the column after 10 days of treatment. Biomass has grown nearly to the maximum. The slight oxygen deficit, however, (the slight break in the oxygen curve), indicates that some small amount of soluble substrate remains. Figure 10 shows a close-up view of the extremely small amount of soluble substrate remaining (trillionths of mg/l's) that accounts for the oxygen deficit. After 20 days of flushing (figure 11) there is no oxygen deficit and all substrate is completely depleted from the column. (Biomass will remain at that (20 day) level, as endogenous decay is not included in the growth equations.)

Figure 12 shows a close-up view of the oxygen deficit after 10 days as compared to the fully oxygenated column at 20 days. The slight oscillations at 20 days are an artifact of the solution method converging on the average level of C_o throughout the column. Figure 13 shows this entire elution process as a function of time (up to 11.6 days, the time required to flush the last of the soluble substrate out of the column). The noticeable curve in figure 13

is the equilibrium curve of excess substrate. (The dissolution rate of excess substrate dC_{xs}/dt is nearly zero just after start-up (when biomass concentrations are too small to convert significant amounts of substrate) and during anaerobic column conditions (where biomass growth is zero). The early change in C_{xs} comes about it dissolves to meet the equilibrium conditions (i.e. to bring soluble substrate up to the theoretical level of C_s throughout the column). The smoothly curved portion of the C_{xs} "elution" curve indicates the change in C_{xs} due to degradation of soluble C.

Figure 14 shows a closer view of the soluble substrate, biomass, and oxygen curves as a function of time. The oxygen curve shows the initial oxygenation of the column (during the first pore volume) from 3.0 mg/l to about 10.3 mg/l. The oxygen concentration doesn't reach saturation levels due to utilization by biomass. Biomass growth is exponential (according to Monod kinetics) but is inhibited by a lack of oxygen for a time during column operation. Biomass levels off at about 12 mg/l, in accordance with the values of y and y_o .

It is important to note that biomass and excess substrate are not actually eluting from the column. These are stationary phases. The values merely represent what is in equilibrium with the oxygen and soluble substrate curves at any time.

Figures 13 and 14 show the equivalent information at the column mid-point after 5.8 days. At the mid-point the column is still operating aerobically, and biomass is in an exponential growth phase.

Using information from figure 11 (concentration/depth profiles at 20 days) it is possible to estimate the proportion of C (present as excess phase and water phase) that is degraded by biomass (as opposed to eluted out of the column). A rough estimate gives the percentage of substrate degraded as 87% of total. This implies very aggressive

degradation, which is not surprising given the assumption of suspended media. (Kinetic constants for biofilms would likely decrease the rate of degradation, and allow more substrate to pass.)

4b. Application to the Reilly Site Treatability Study

As noted above, the Reilly site soil column is very similar in design and operation to the theoretical "base case" example. To account for the major difference, retarded mass transfer from the excess phase, KSA was assigned a value of $0.0004 \text{ cm}^2/\text{mg}$, and N was assigned a value of 0.33. For our system, this approximates mass transfer from a non-film phase (near cubical); and effectively lowers the rate of dissolution in accordance with experimentally observed values. (The actual values were still lower than those obtained in the model, however, these gave satisfactory results.)

More significantly, the Cs value was lowered to 1 mg/l (Ci was lowered to 0.200 mg/l.) [The Cs represents the equilibrium value of C possible in the column during dissolution.] Figure 17 shows the resulting dissolution profile of substrate from the excess phase over the column length. (As a comparison, thin film dissolution is nearly instantaneous).

Other changes to the input parameters from the base case include altering the incoming oxygen level to 9.20 mg/l, in accordance with saturation levels at room temperature, and setting the Rc value at 2.00, representing substrate less likely to bond reversibly with soil particles. [Again in accordance with experimentally predicted values.]

The model results are shown in figures 18 through 25. Figure 18 shows column concentrations after one pore volume has been flushed. Note the excess substrate is not

dissolving nearly as quickly as in the "base case". (Monod coefficients and flow characteristics remain the same between the two cases.) Figure 19 shows that after 5 days of flushing this column, enough substrate has dissolved to begin to a significant biomass population (with the concomitant oxygen deficit). In figure 20 (after 10 days of operation) a large biomass population is present throughout most of the column, and the last 10 cm of the column is nearly anaerobic. Figure 21 shows concentration profiles after 20 days of column operation. Here biomass growth is (unrealistically) high. There is still excess substrate left in the column after 20 days (due to restricted mass transfer to the water phase). The presence of oxygen (8.9 mg/l) at the column outlet indicates that biomass growth is substrate-limited.

Figure 22 shows the "elution" curves (concentration versus time) for each species through 20 days. Excess substrate is nearly depleted, while biomass continues to grow (growth is substrate-limited). Oxygen, initially increased in the column (during the first pore volume), then slowly depleted, begins to "return" to the column after 10^6 seconds as demand slows. This relationship is easier to see in the close-up, figure 23.

Figure 24 shows the model operating over 25 days with the corresponding further decrease of soluble substrate (though it is still not zero), and incremental increases in biomass and oxygen (as compared to 20 day values). Figure 25 shows the "elution" curves at 25 days. Note the very gradual decline of excess substrate over time (and the correspondingly gradual increase in biomass). Compared to the base case, the column is nearly anaerobic for a shorter period of time, with higher levels of oxygen present at the end of the operating period. Biomass growth is more steady throughout.

4c. Application to Plume Management - Treating a Spill with In-Situ Biodegradation

Throughout this example the kinetic constants and flow parameters are consistent with those in the initial base case.

To model a migrating spill, the "spill" is first described over the length of the excess substrate (L_{cxs}). Then, the length of the column ($L > L_{cxs}$) is adjusted to represent the extent of the resulting plume. The time over which the model runs is adjusted (as before) to coincide with the center of mass movement of the plume. [Because $R_c = 10$, tracking the center of mass overestimates the arrival of the plume front.]

In each subset of data ("untreated plume", "aerated plume", and "H₂O₂ treated plume") the original spill is six inches of 100 mg/l excess substrate. (This small amount of contamination is required to allow for reasonable computation times: about 25 minutes of CPU time/run.)

The first case is that of the "untreated plume". Here, the spill, using the same kinetic parameters as in the "base case", migrates through virgin soil containing microbes (that are assumed to be acclimated to the incoming waste). The microbes degrade the spill with the oxygen available in the flushing water. (It is assumed to have 11.4 mg/l dissolved oxygen coming into the six inch contaminated zone.) Figures 26 through 29 track the movement of the plume from shortly after the spill occurs until it is (almost) remediated six days later.

Figure 26 shows the plume moving through the first 3 feet of soil. The original excess substrate is solubilized almost immediately (so it does not appear here), while the "plume" of soluble substrate is highly visible "moving" through the column. Oxygen depletion and

biomass accumulation are evident. Figure 27 shows the plume moving through 6 feet of soil. By now, the peak is substantially reduced due to degradation, adsorption, and dispersion ($D_c = 0.010 \text{ cm}^2/\text{sec}$). The oxygen depletion corresponds to maximum biomass growth. Figure 28 tracks the plume through 12 feet of soil. The soluble substrate is now almost completely degraded (about 0.4 mg/l remain). Oxygen has reached its minima (1.1 mg/l) at about 9.5 feet from the original spill site, and is beginning to recover as Monod growth becomes substrate-limited.

Figure 28 shows a the "spread" of the plume as it migrates over 3, 6, and then 12 feet of soil. [The decrease in peak height is also due to removal of substrate by biodegradation.]

Figures 29 through 35 model the plume (with the same kinetic parameters) when aerated using a hollow fiber membrane, or other suitable technology, that will allow maximum theoretical oxygen supersaturation concentrations (say, 40 mg/l) in water to contact the plume at the source. The key assumptions here are that maximum supersaturation can be achieved, and that the technology delivers this value directly to the groundwater in-situ, without substantially increasing the flowrate of water past the plume.

Figure 29 shows the aerated plume moving through 3 feet of soil. As can be seen in figure 30 there are negligible benefits to "air sparging" at 3 feet. (This figure overlays figures 29 and 26.) Figure 31 tracks the aerated plume through 6 feet. The substantial oxygen drop indicates biomass demand. Figure 32 shows the relative benefits of the aeration after 6 feet. (Again, compared to "untreated plume".) Figures 33 and 34 show this plume moving through 12 feet, and the final relative benefit of aerating at 12 feet, respectively. (Figure 34 shows a 90% decrease in the mass of migrating chemical at 12 feet using aeration, albiet small amounts of chemical are involved at that point.) Figure 35 shows the familiar spread of the plume through 3, 6, and 12 feet.

These figures show that aerating the contaminated plume had a slight effect on removal. While it is difficult to determine whether this small effect could be cost-effective on a much larger scale, the results are promising. It should be noted that our system was expected to show negligible benefits to aeration, as it did not operate anaerobically over the extent of plume even when "untreated". Had the spill been larger or the available oxygen much less, aeration would have had a larger effect. This simulation demonstrated that significant barriers did not exist.

Figures 36 through 40 show the effects on plume management of treating the spill with H₂O₂ solution, assumed at maximum concentrations to deliver 100 mg/l equivalent O₂. The H₂O₂ solution is added via an injection well driven just upstream of the contaminated site. Here the solution must take into account the increase in average linear velocity past the contaminated zone. Hence, v was estimated at 4 feet/day in the first 3 feet past the spill, and 3 feet/day in the next 9 feet.

Figure 36 shows the plume (treated with H₂O₂) moving past the first three feet of soil. Interestingly, the excess substrate phase (the "spill") is still readily observable in the first 3 feet of remediation. The change in velocity has overwhelmed the system, and microbes have not yet degraded enough soluble substrate to force excess substrate to dissolve. Figure 37 shows a close-up comparison of soluble substrate in this scenario versus the "untreated plume". Figure 38 shows the "large" H₂O₂ treated plume moving through 6 feet of soil relative to the "smaller" untreated plume. The difference in the amount of biomass present is also evident. Figure 39 shows the H₂O₂ plume pushing past the 12 feet of soil that contained the untreated and aerated plumes. Obviously, for this system controlling the advective velocity is much more important than delivering extra oxygen to

the center of the plume. This is made clear in figure 40, which is analagous to figures 28 and 35.

Again, it is difficult to predict at what point the benefits of H₂O₂ delivery (faster degradation) would outweigh the risks (increased spread of plume). In this system, oxygen is not limiting to a large degree, so adding H₂O₂ has no real benefit. In a larger, anaerobic system there can be a tremendous upside to this technology.

In general, the results of the plume management studies gave satisfactory results. In comparison with field observations of similarly contaminated soil (Goerlitz, et al, 1985; Ehrlich, et al, 1982; and Borden and Bedient, et al, 1986), it appears that the example level of retardation ($R_c = 10$) may be too high as significant adsorption did not influence retardation of solutes in groundwater transport in situ.

TPMBX2 predicts that in the case of the small, largely aerobic spill little benefit can be obtained by aerating with gas delivery systems, and no benefit can be gained with "liquid" oxygen delivery systems. While this outcome is not surprising, it is instructive. TPMBX2 is a flexible modelling tool for both finite column and plume management studies.

Figure 5

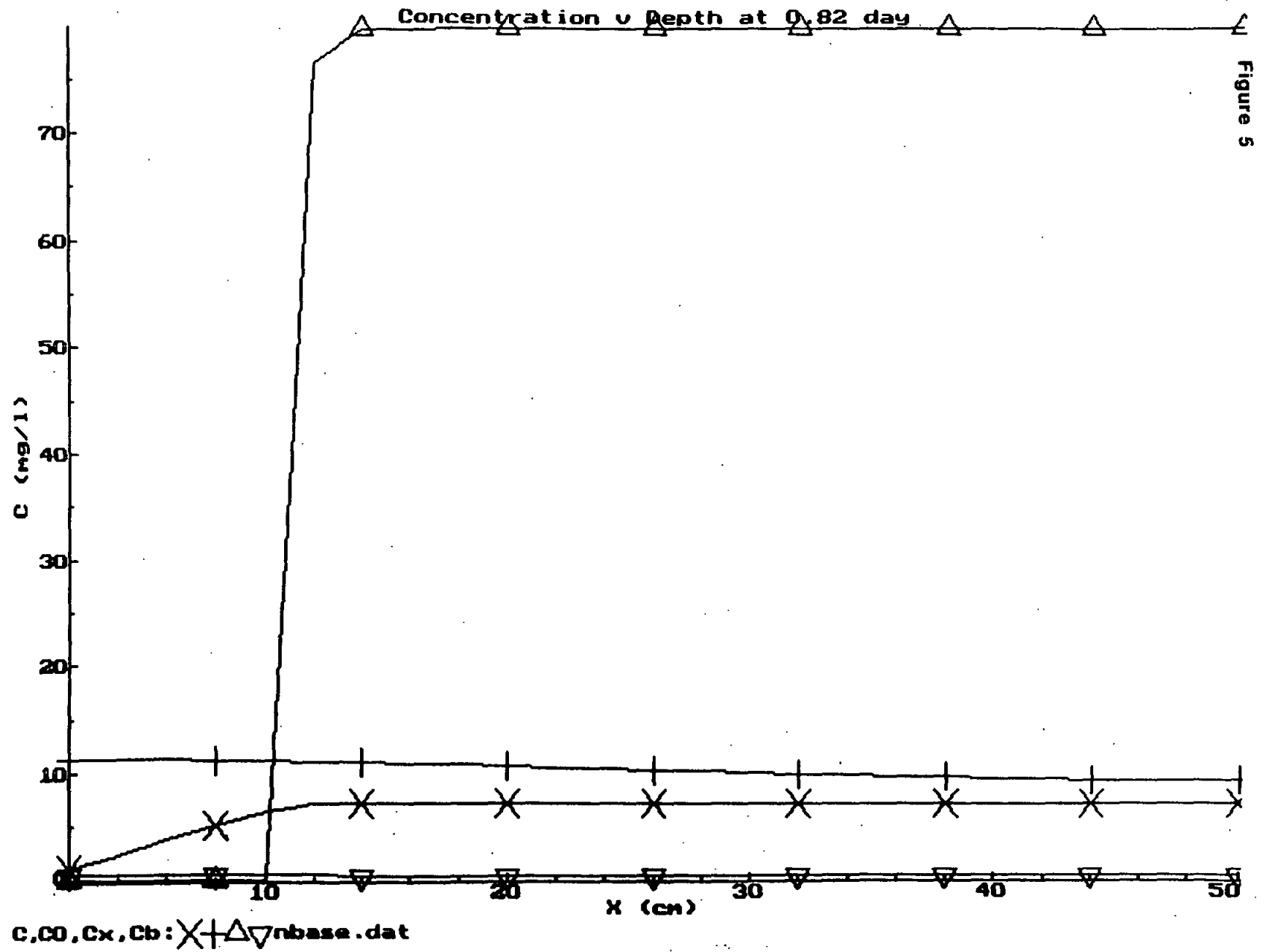


Figure 6

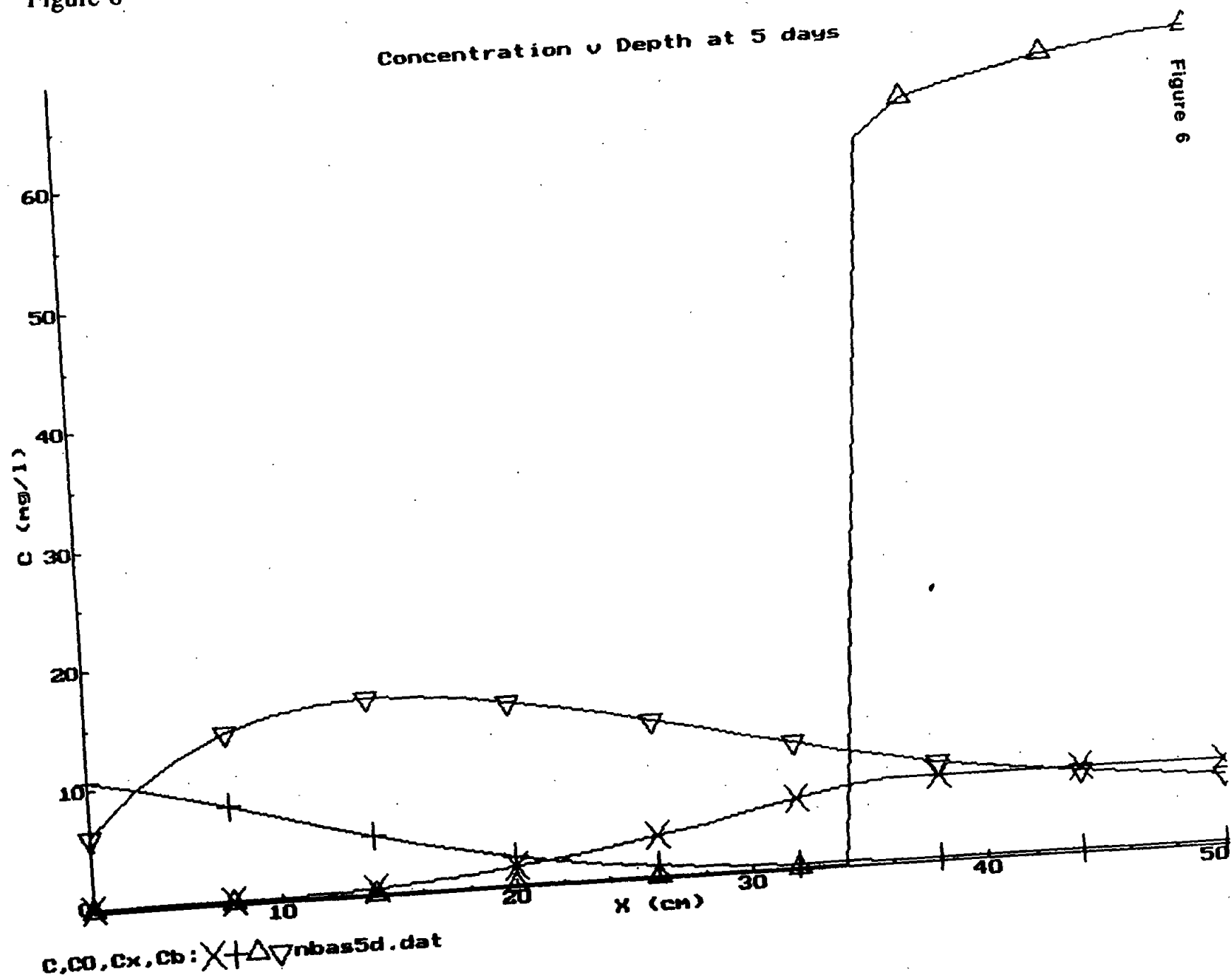


Figure 7

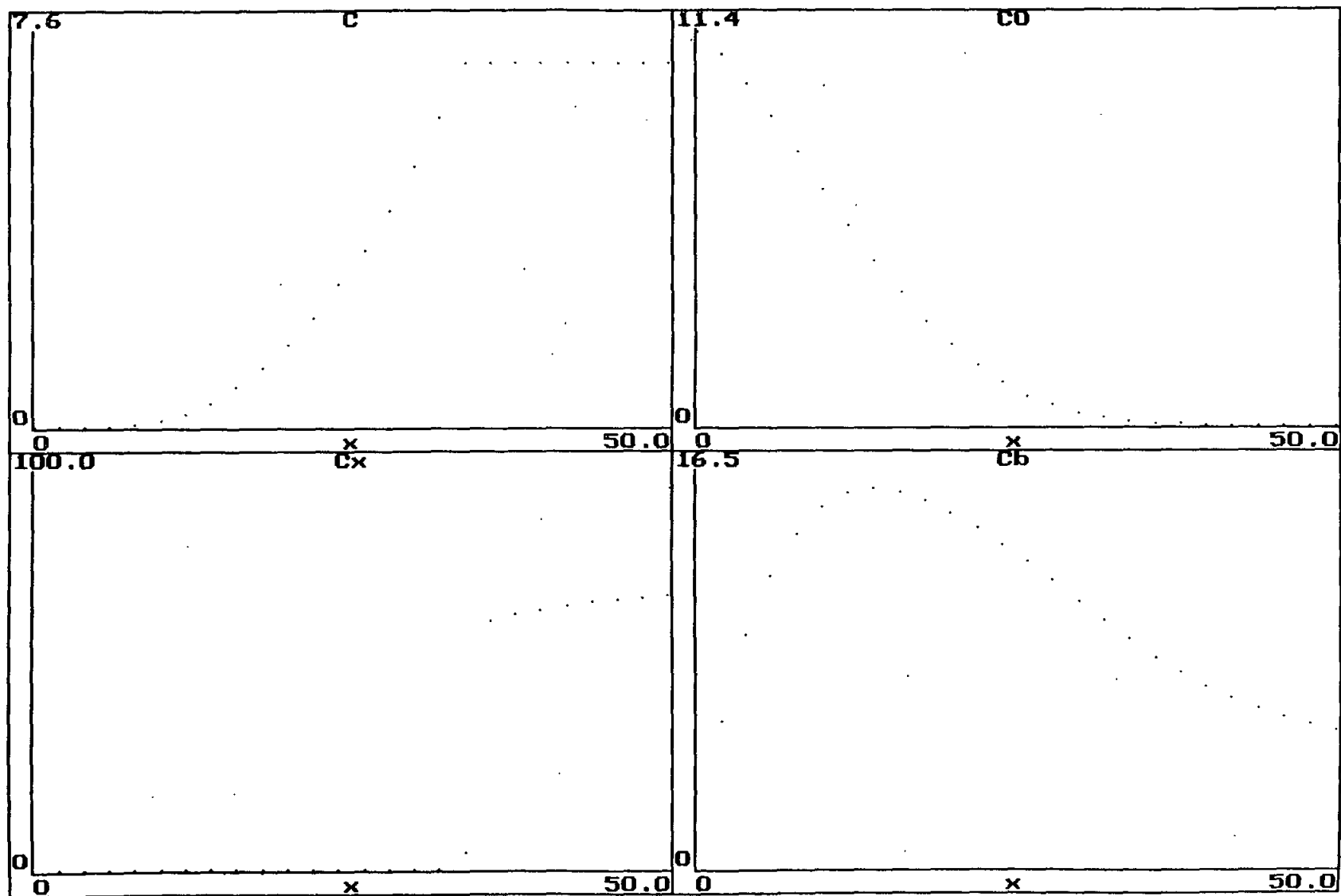


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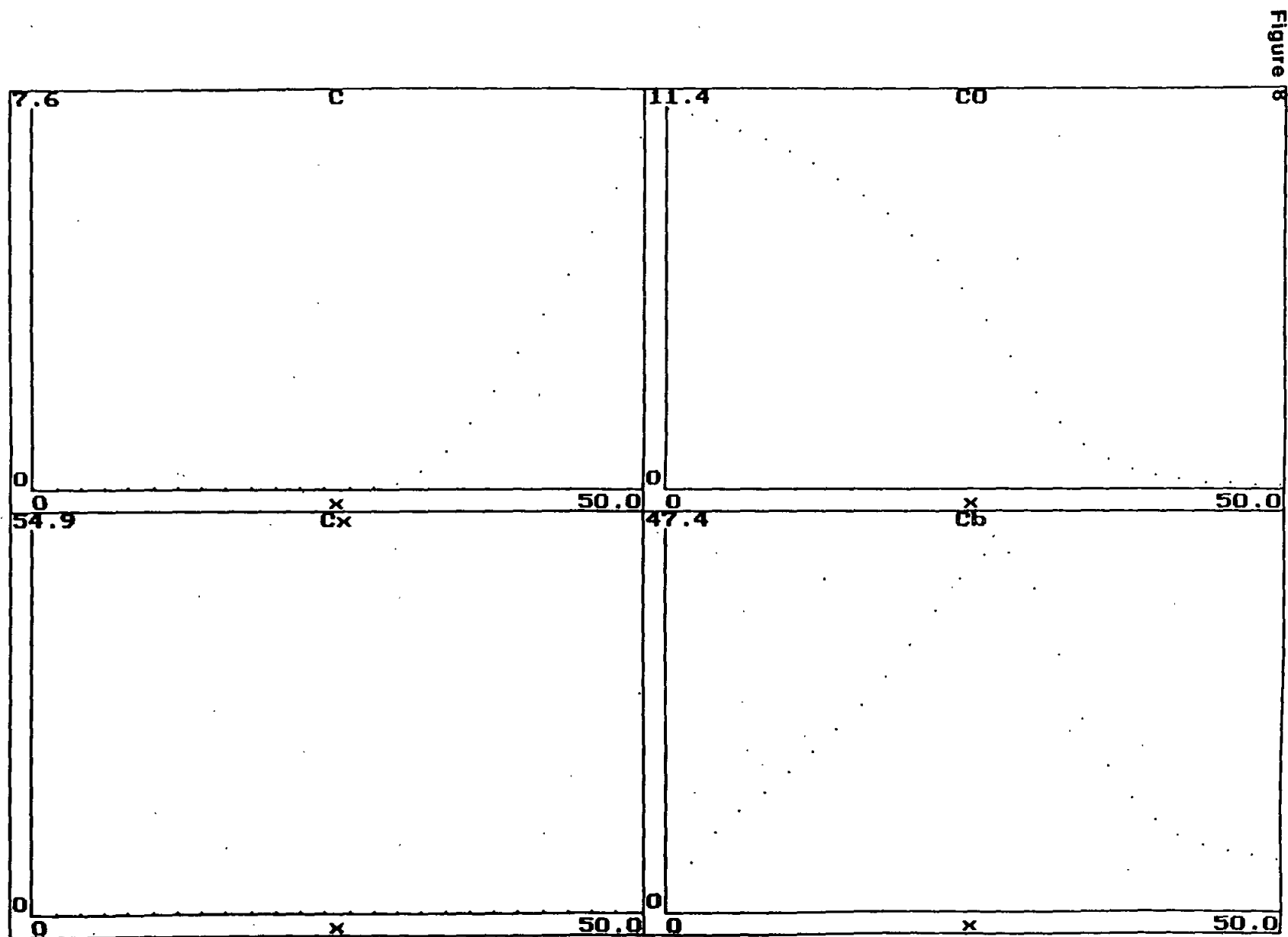


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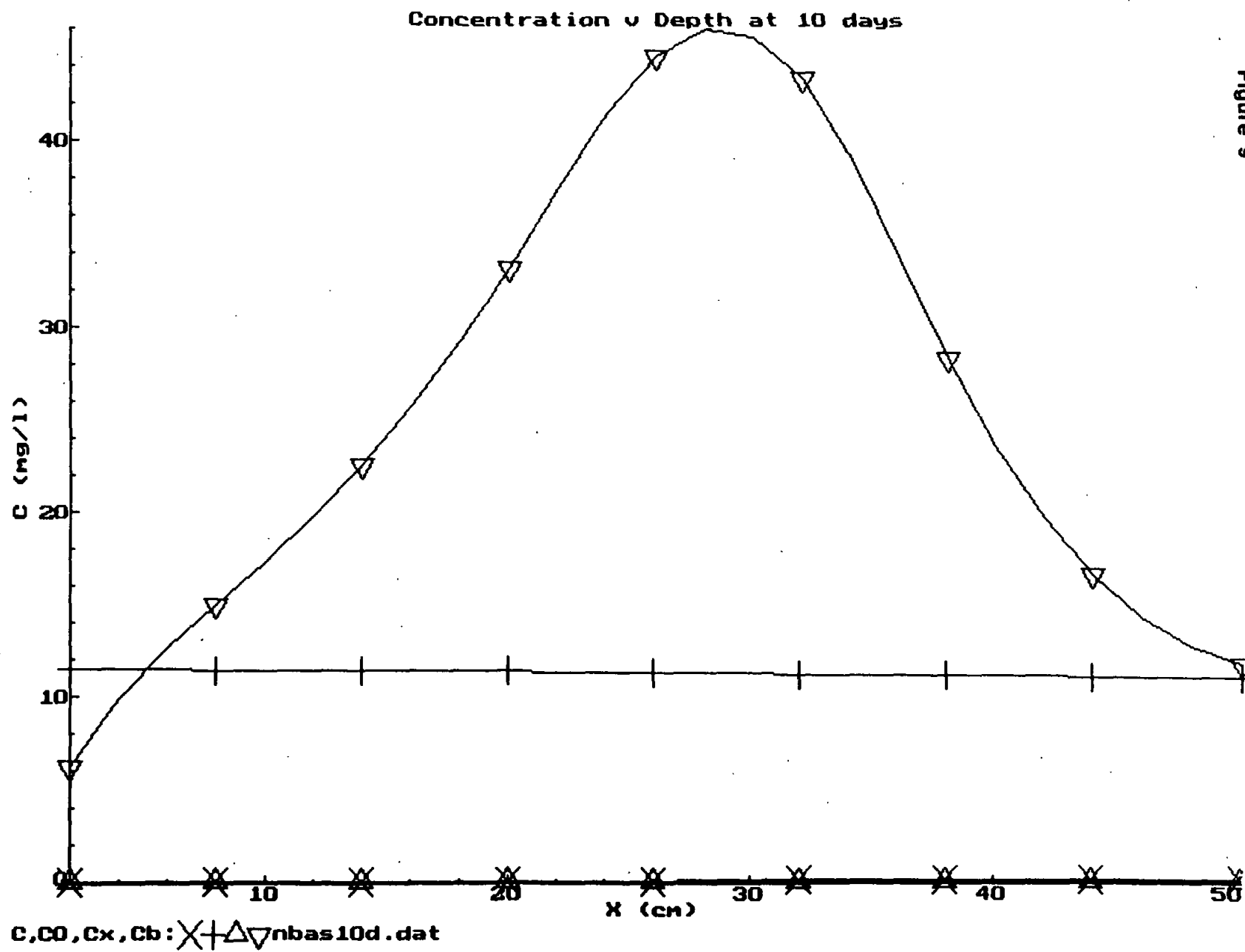


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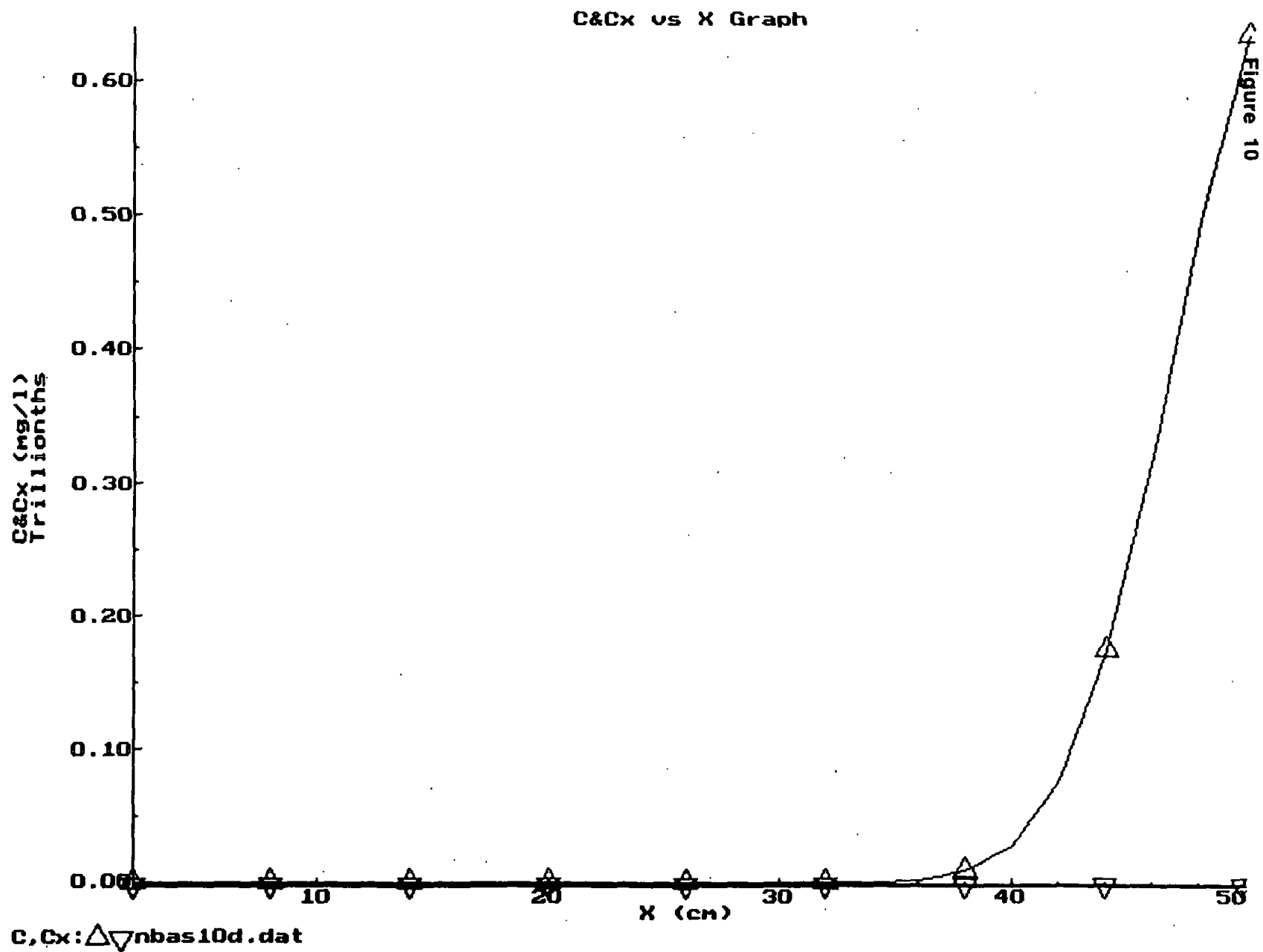


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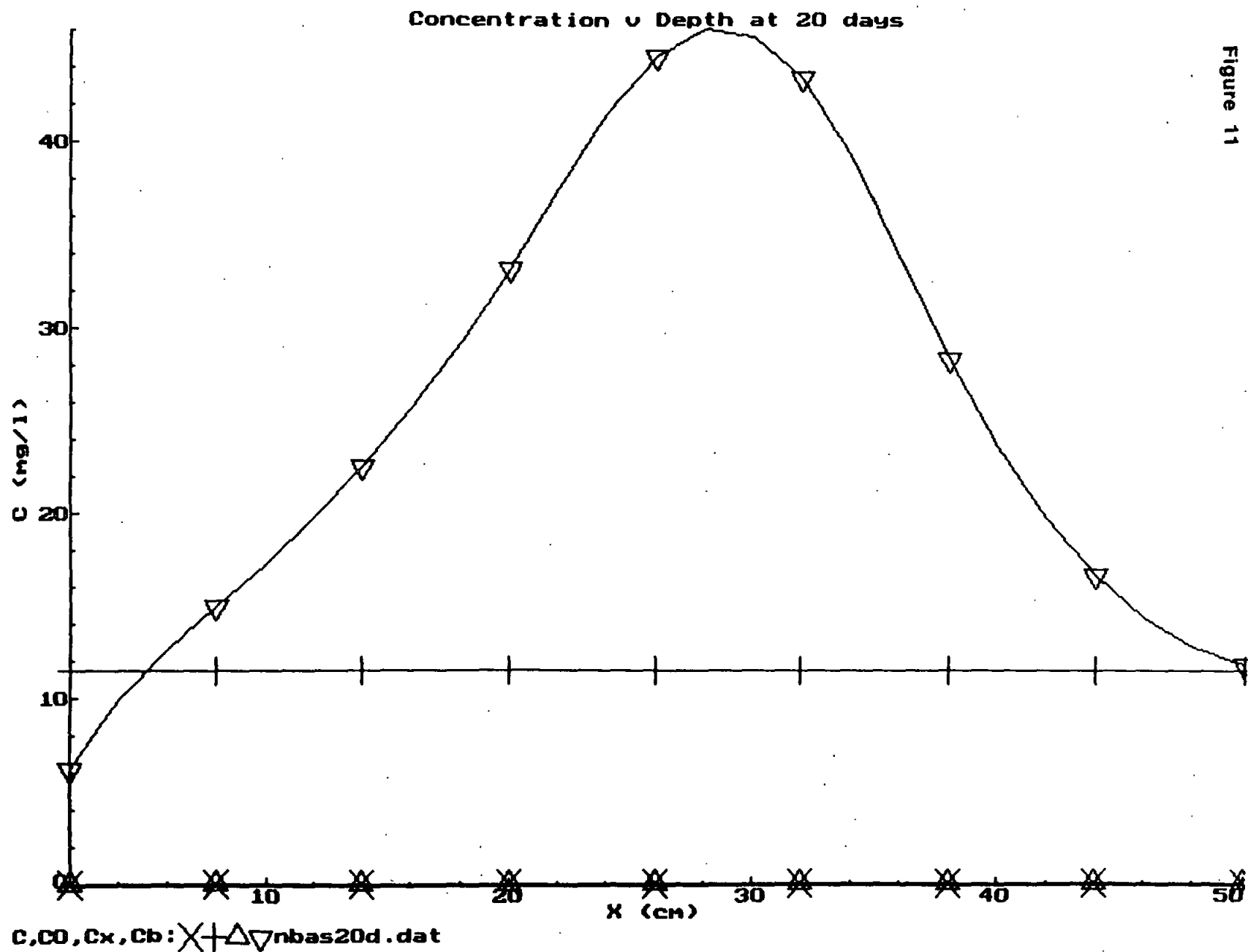


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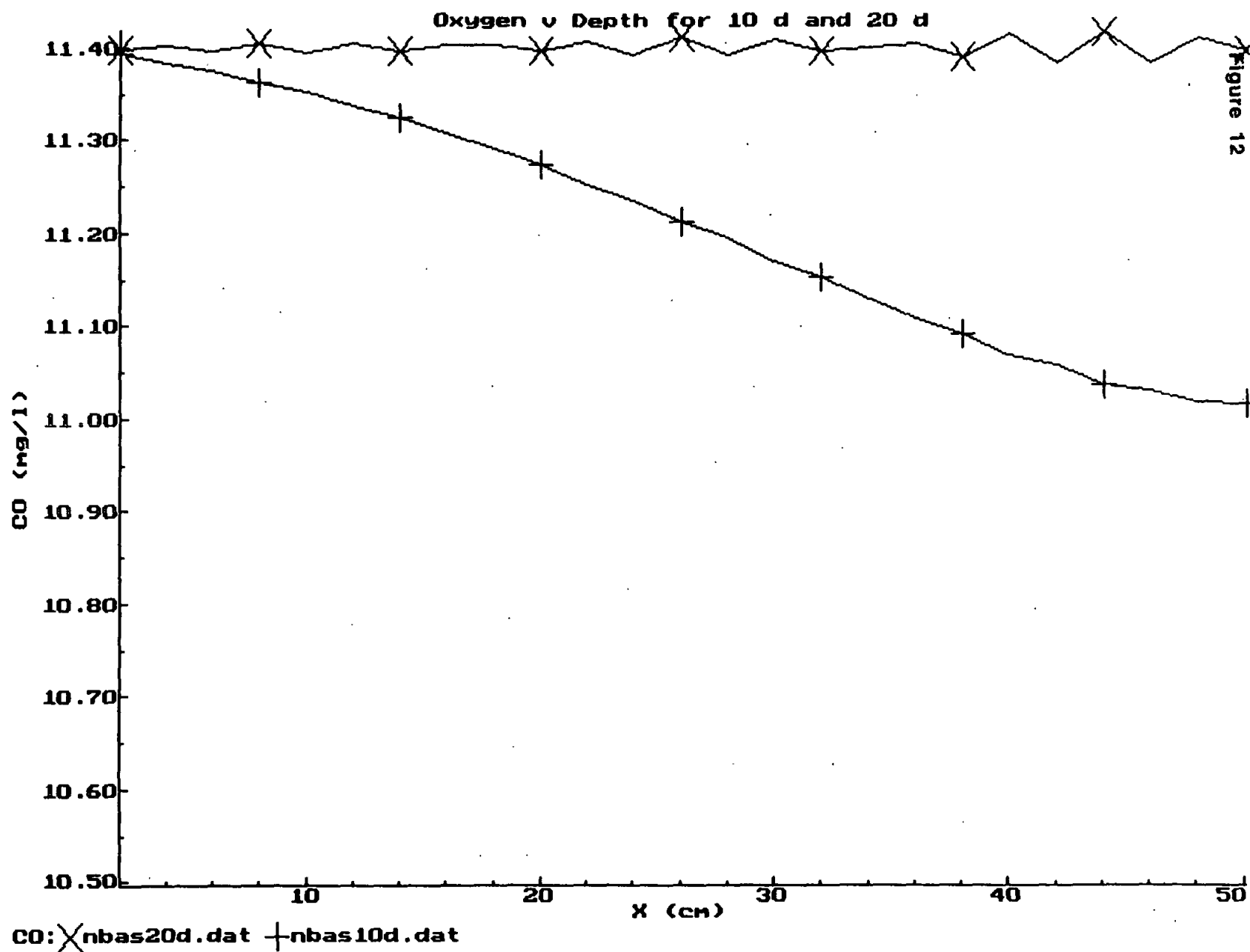


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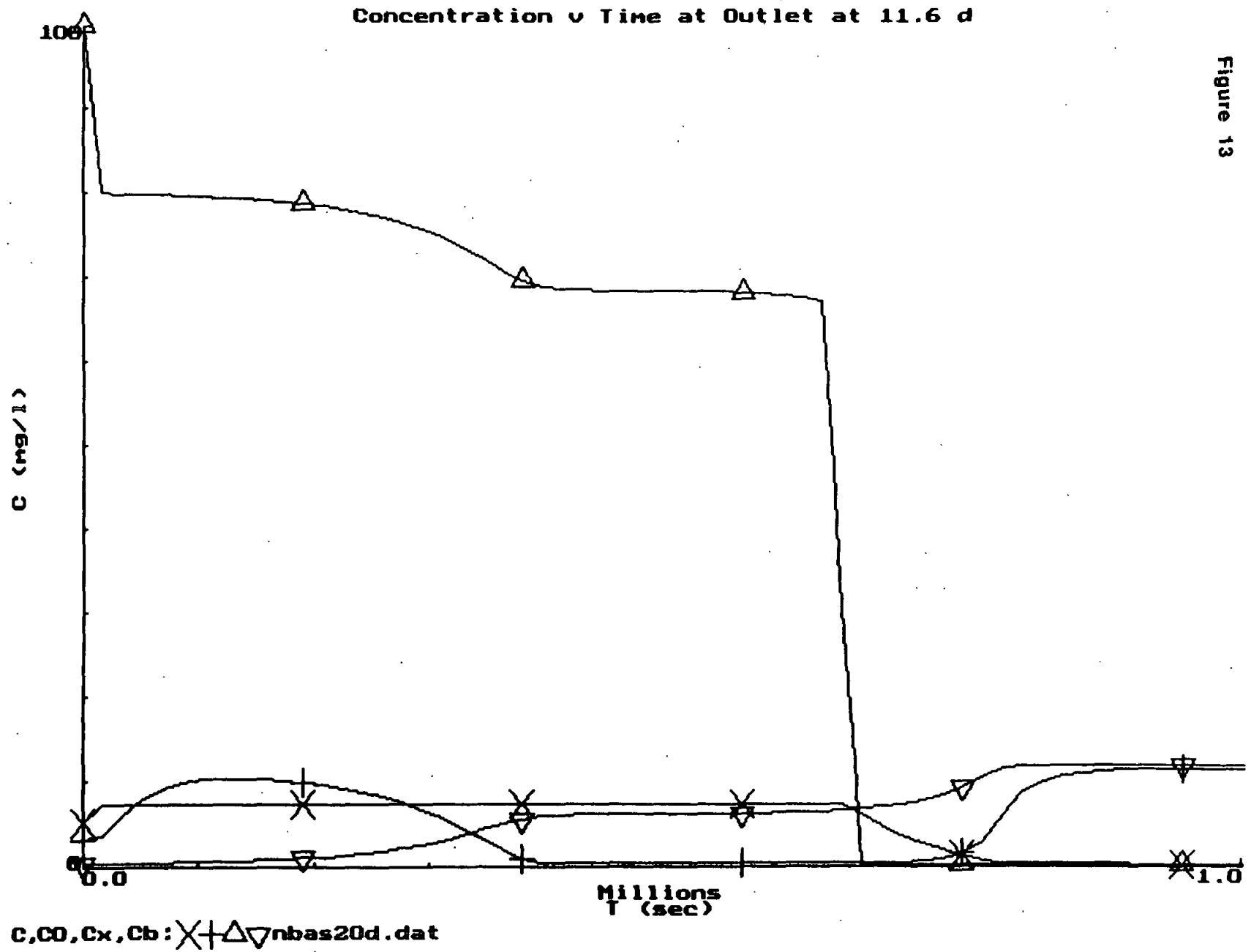


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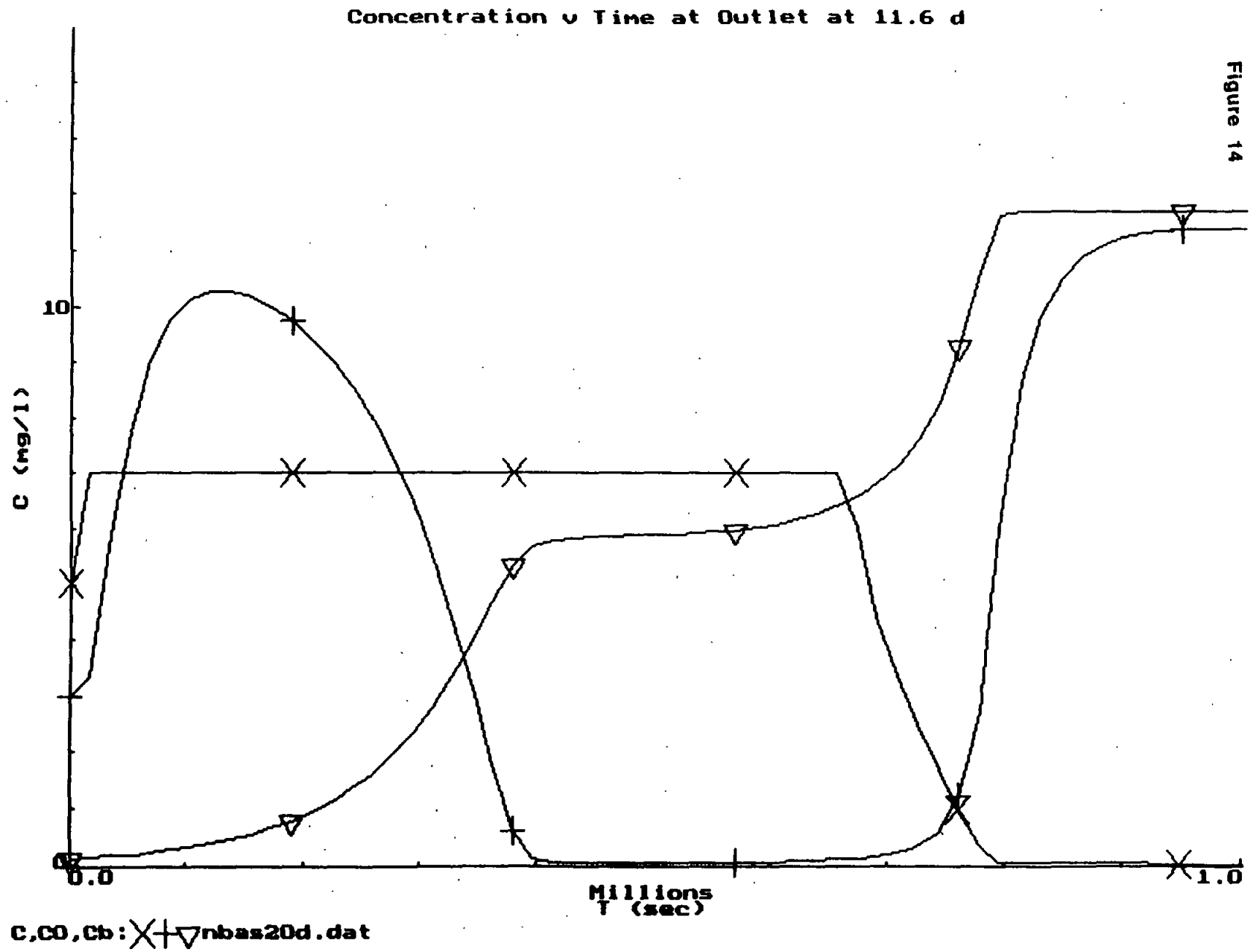


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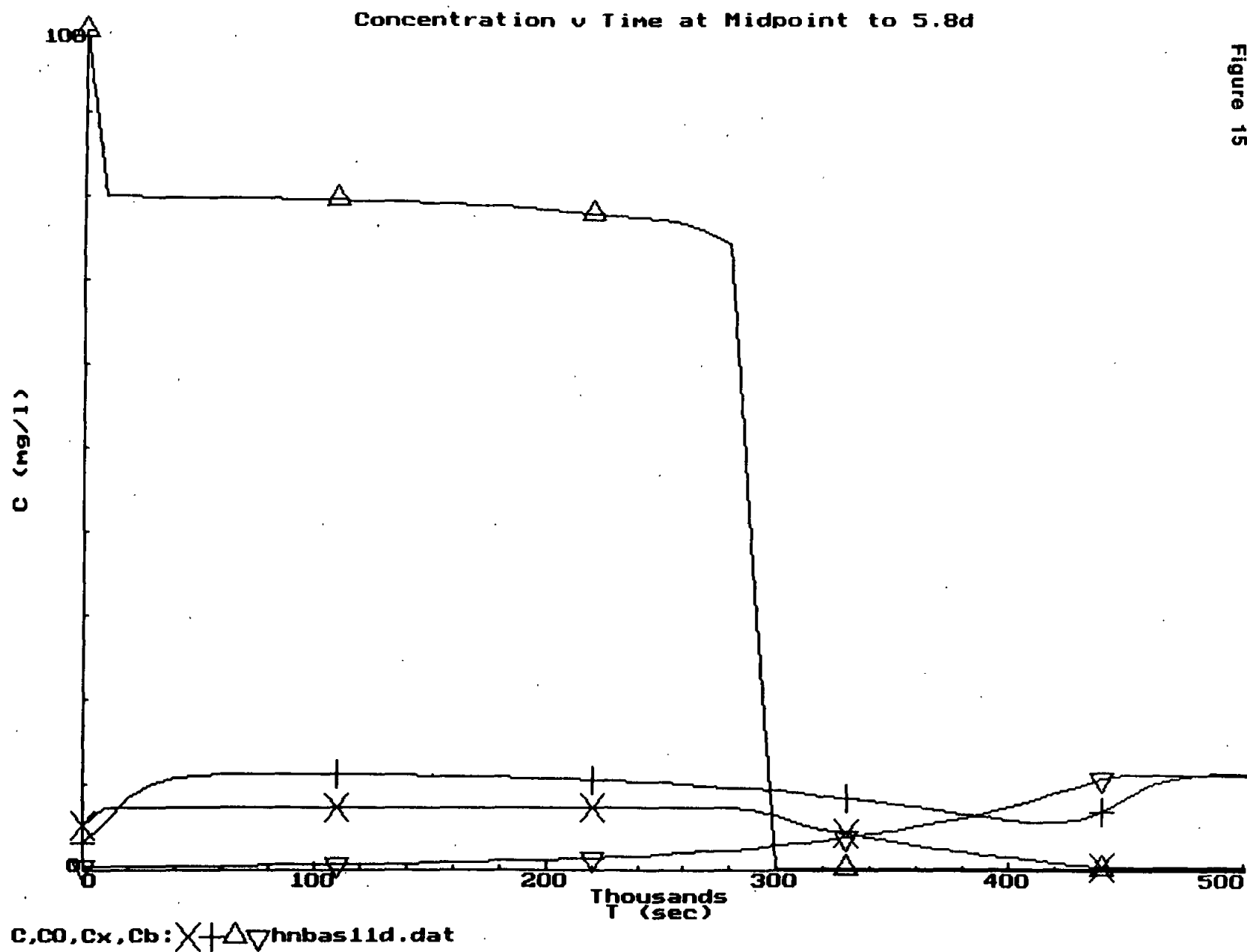


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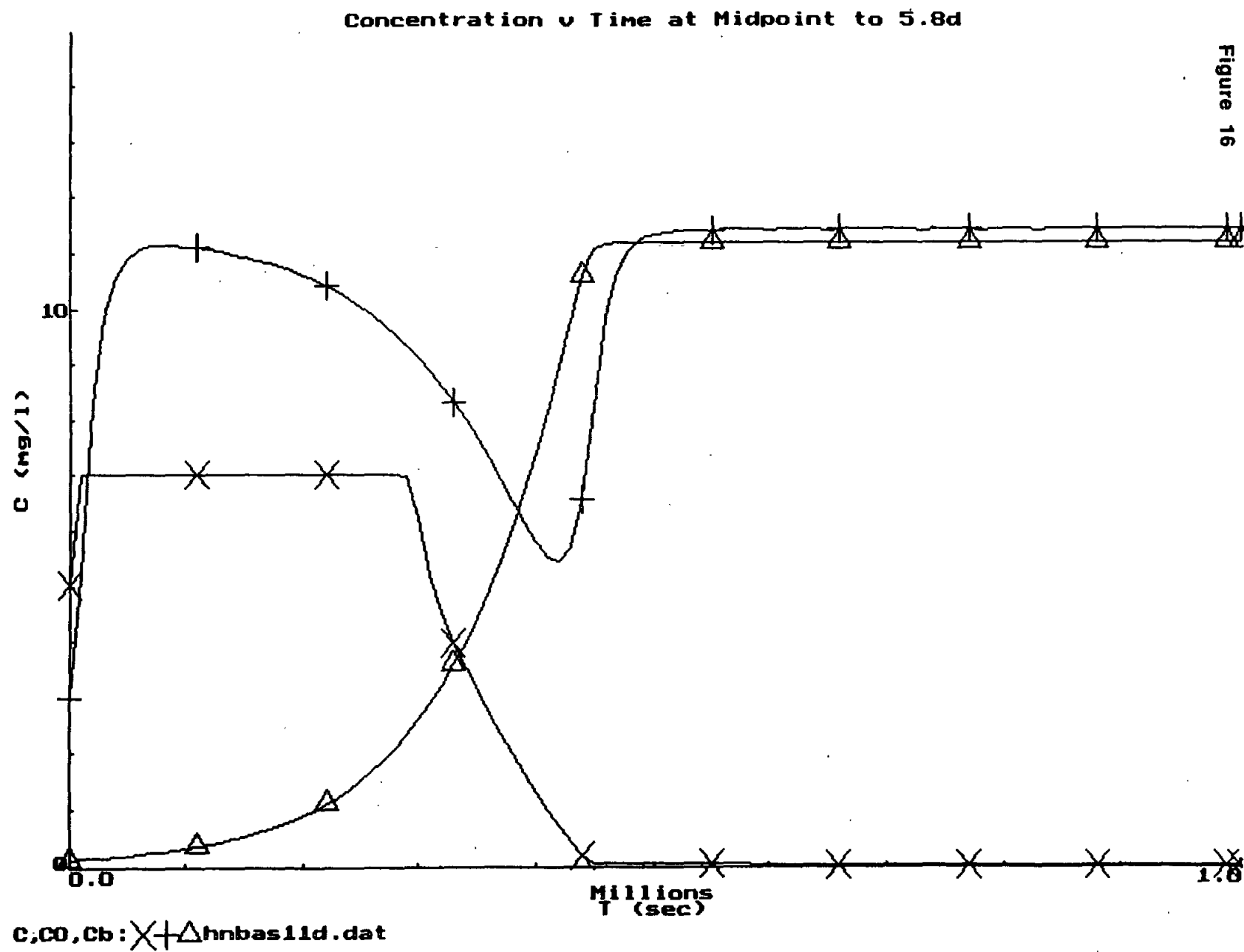


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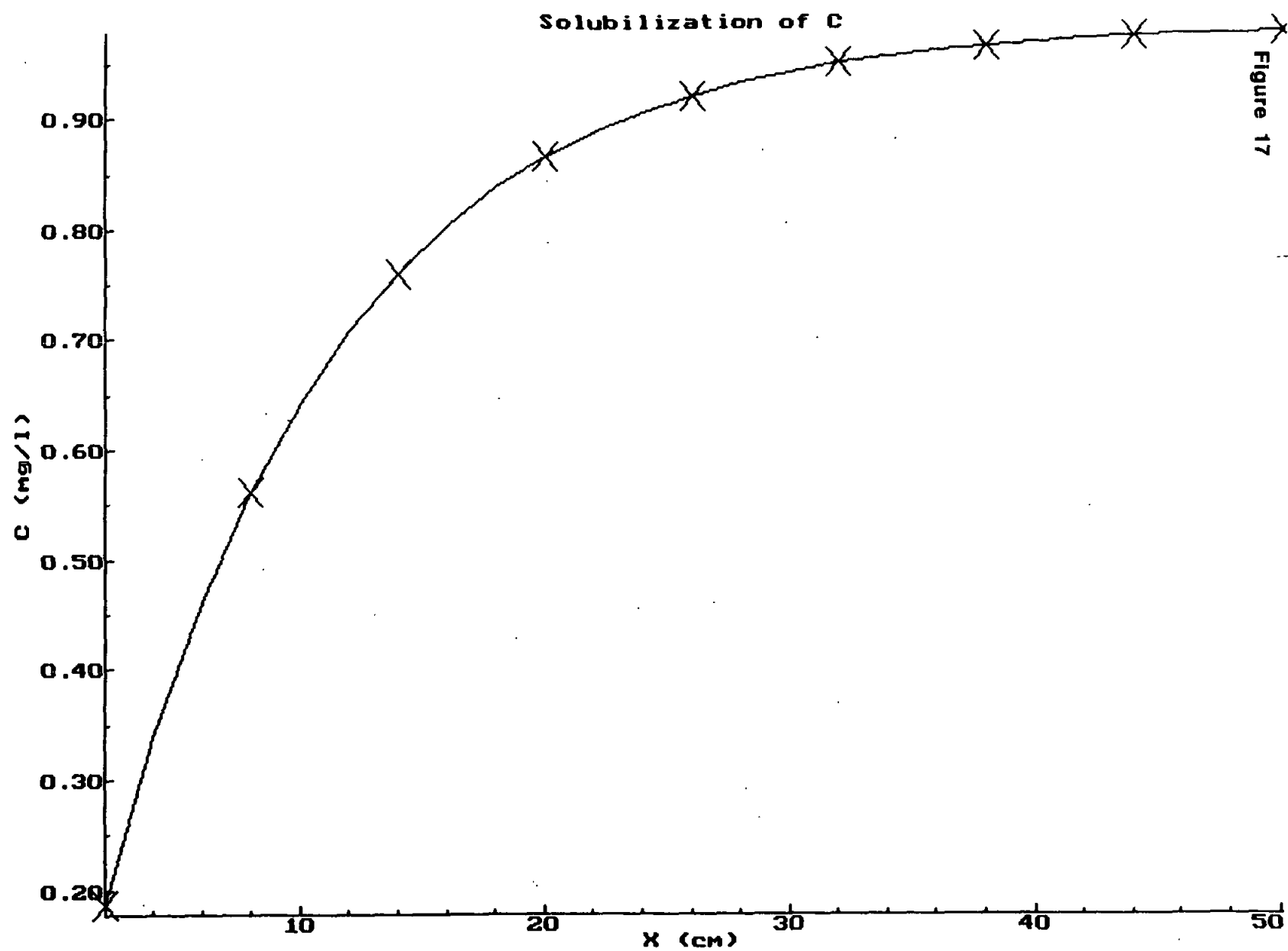


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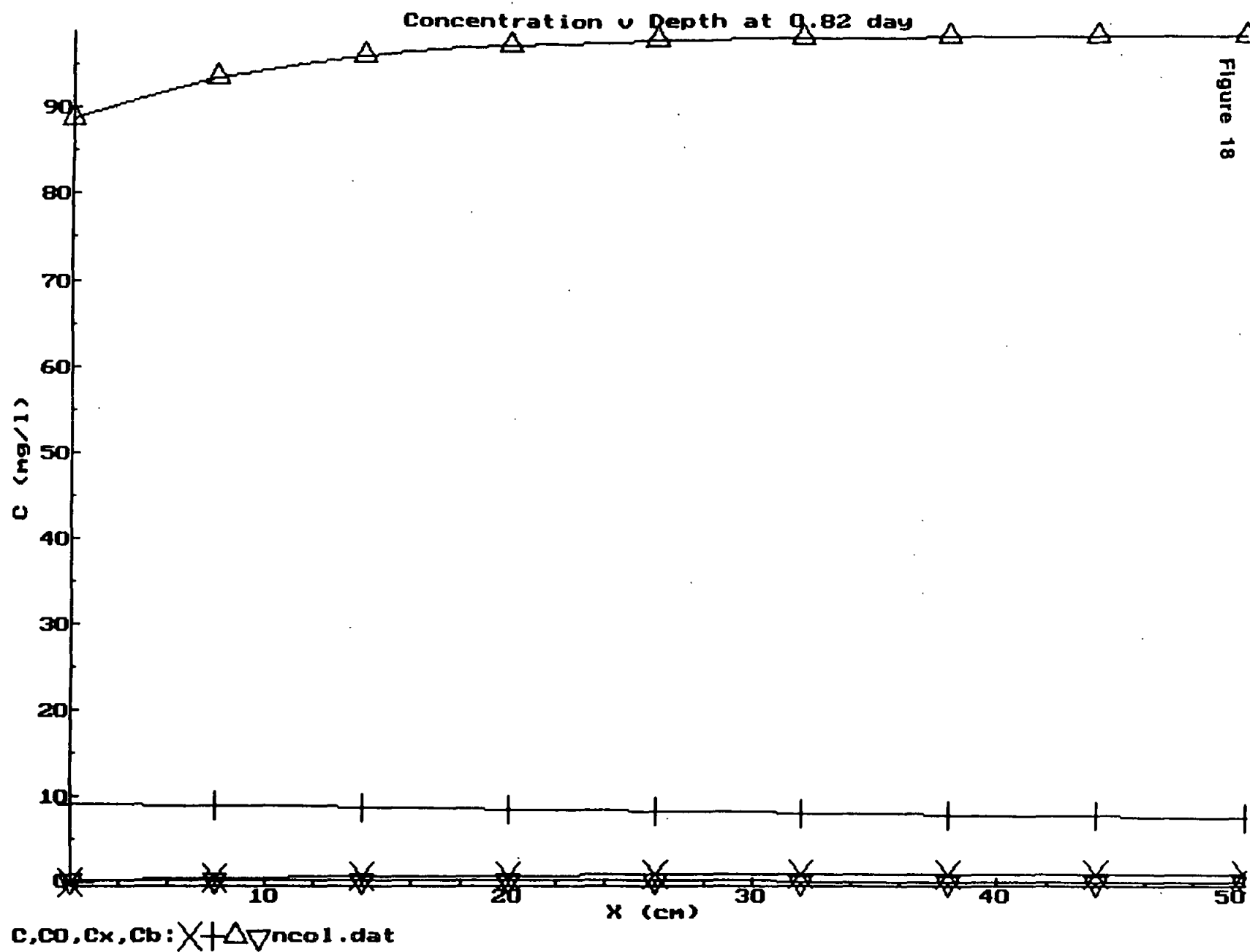


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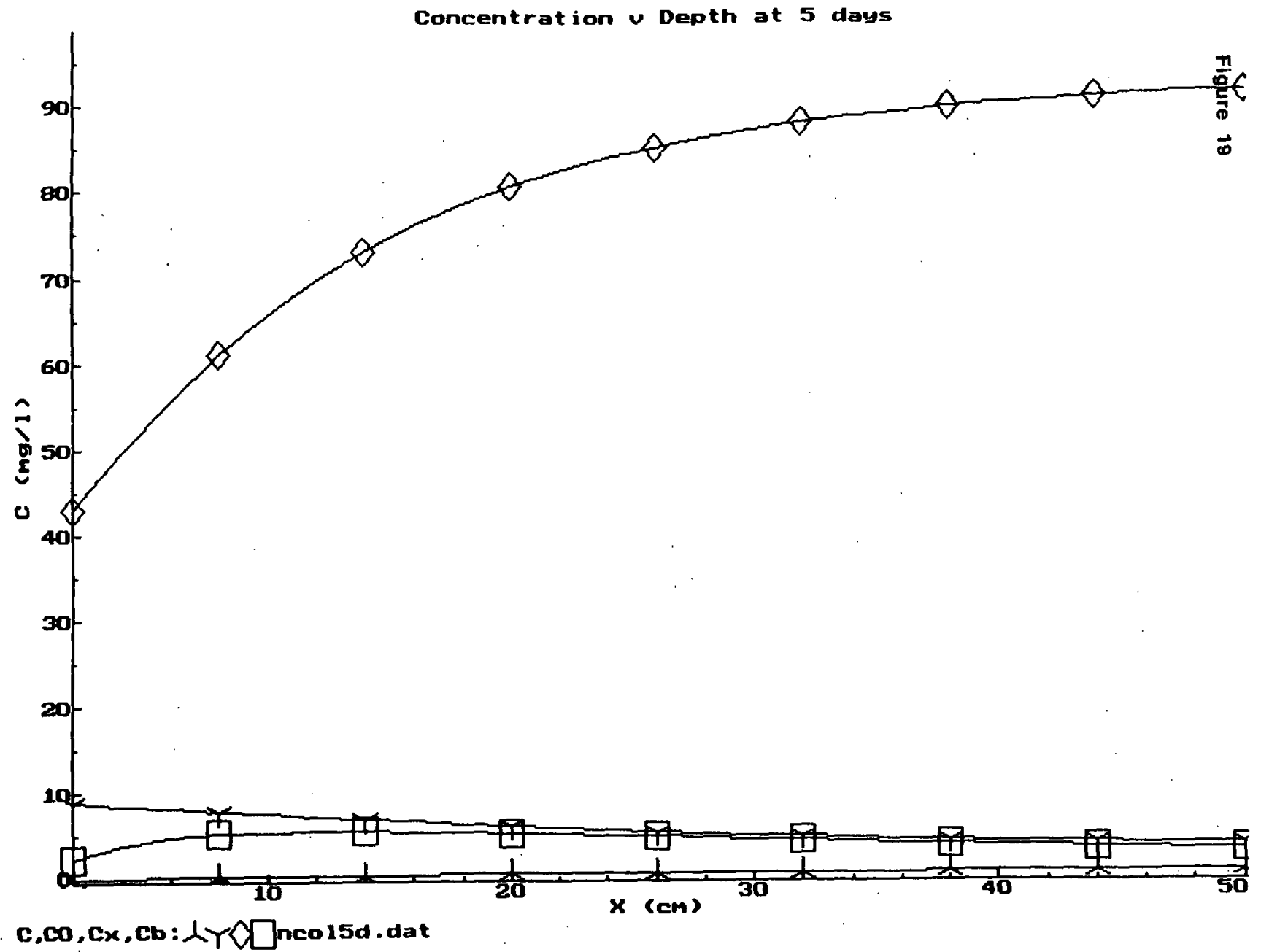


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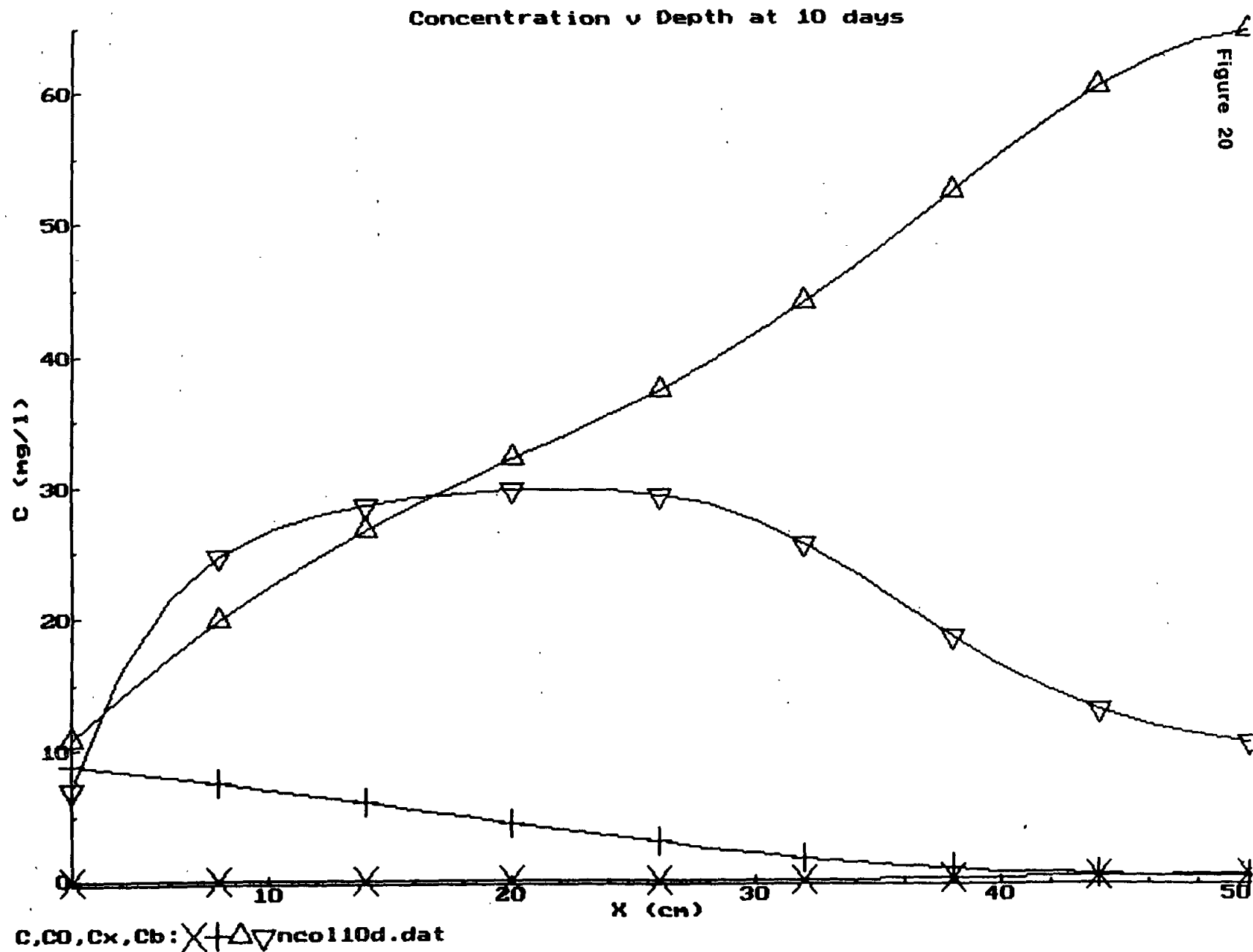


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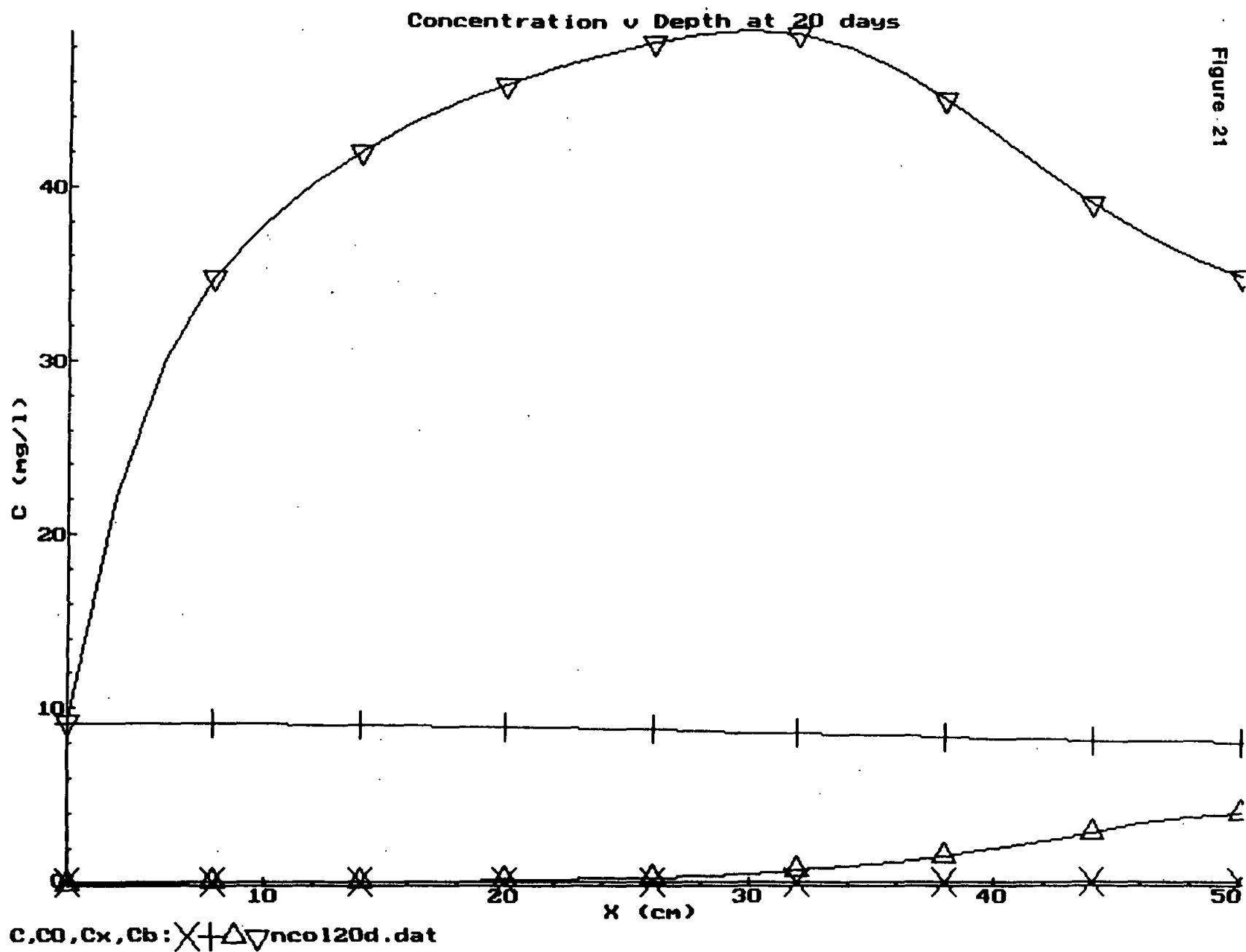


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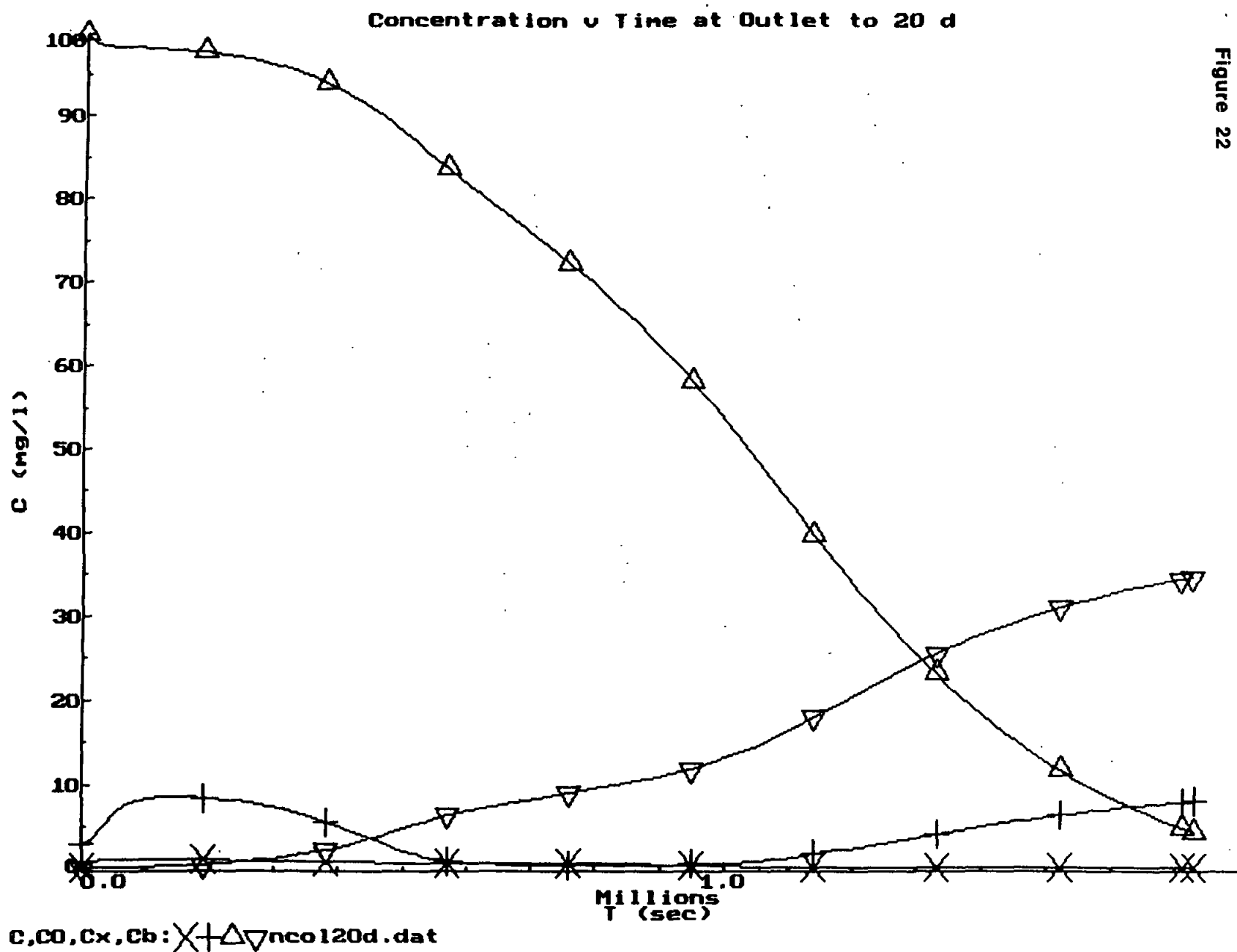


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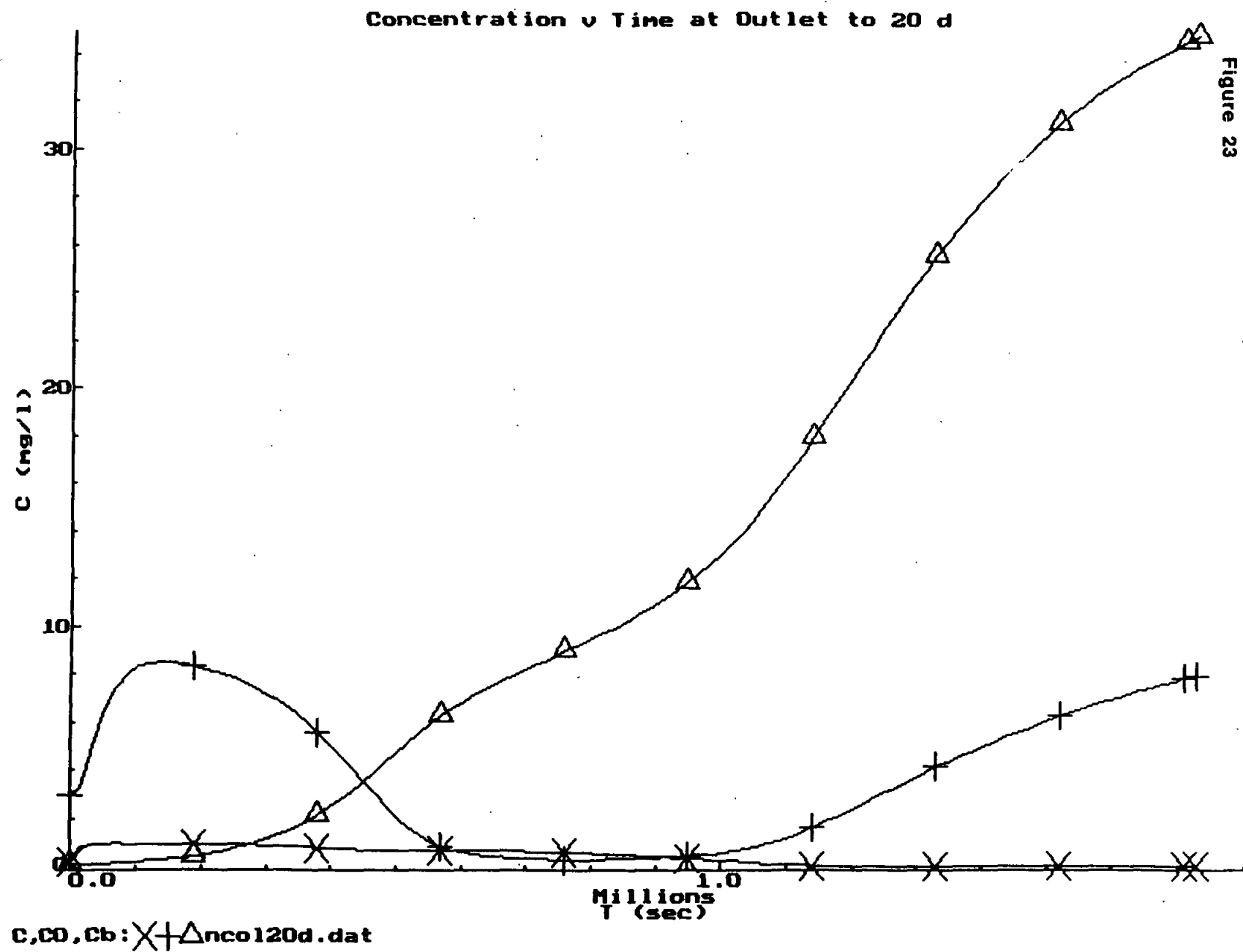


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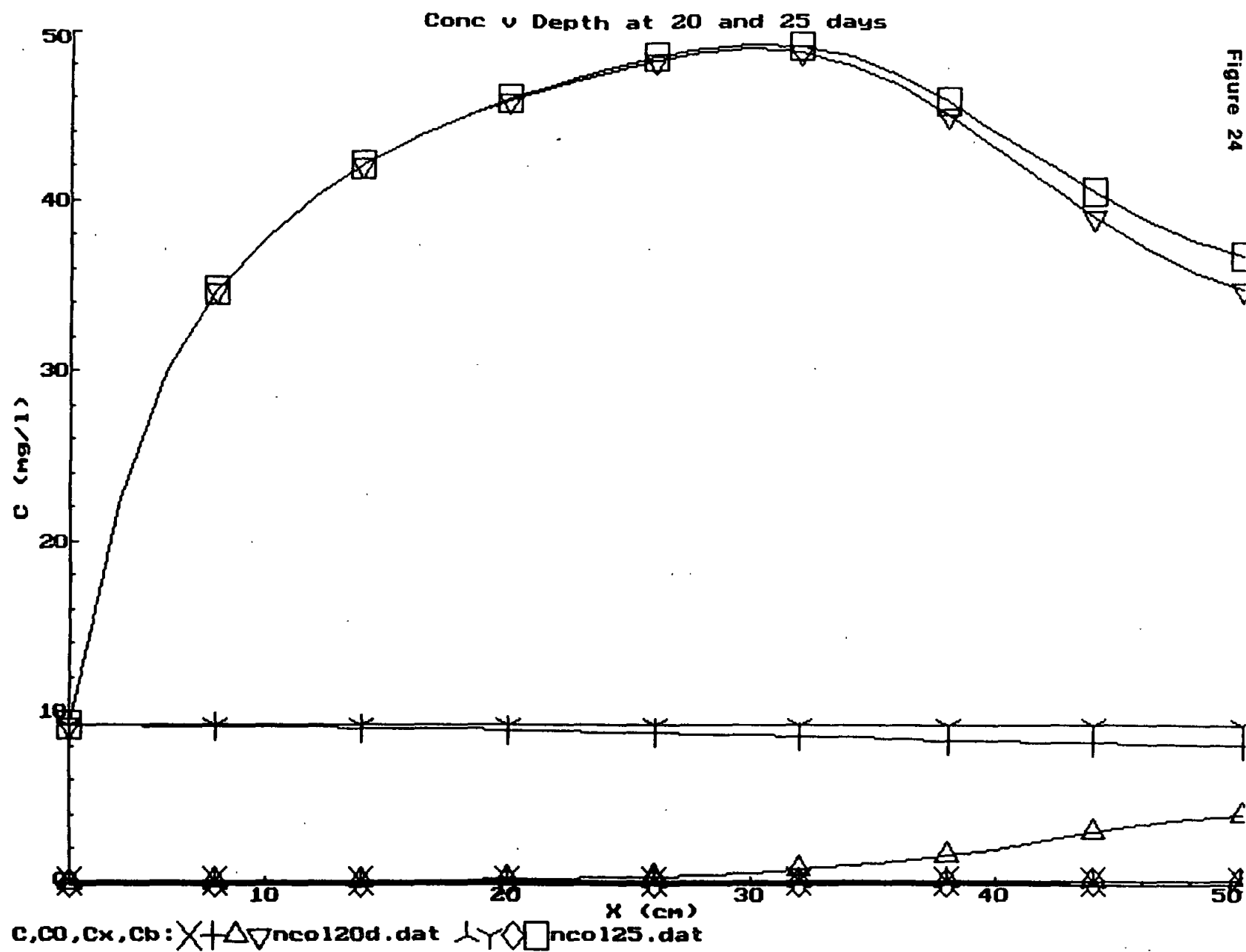


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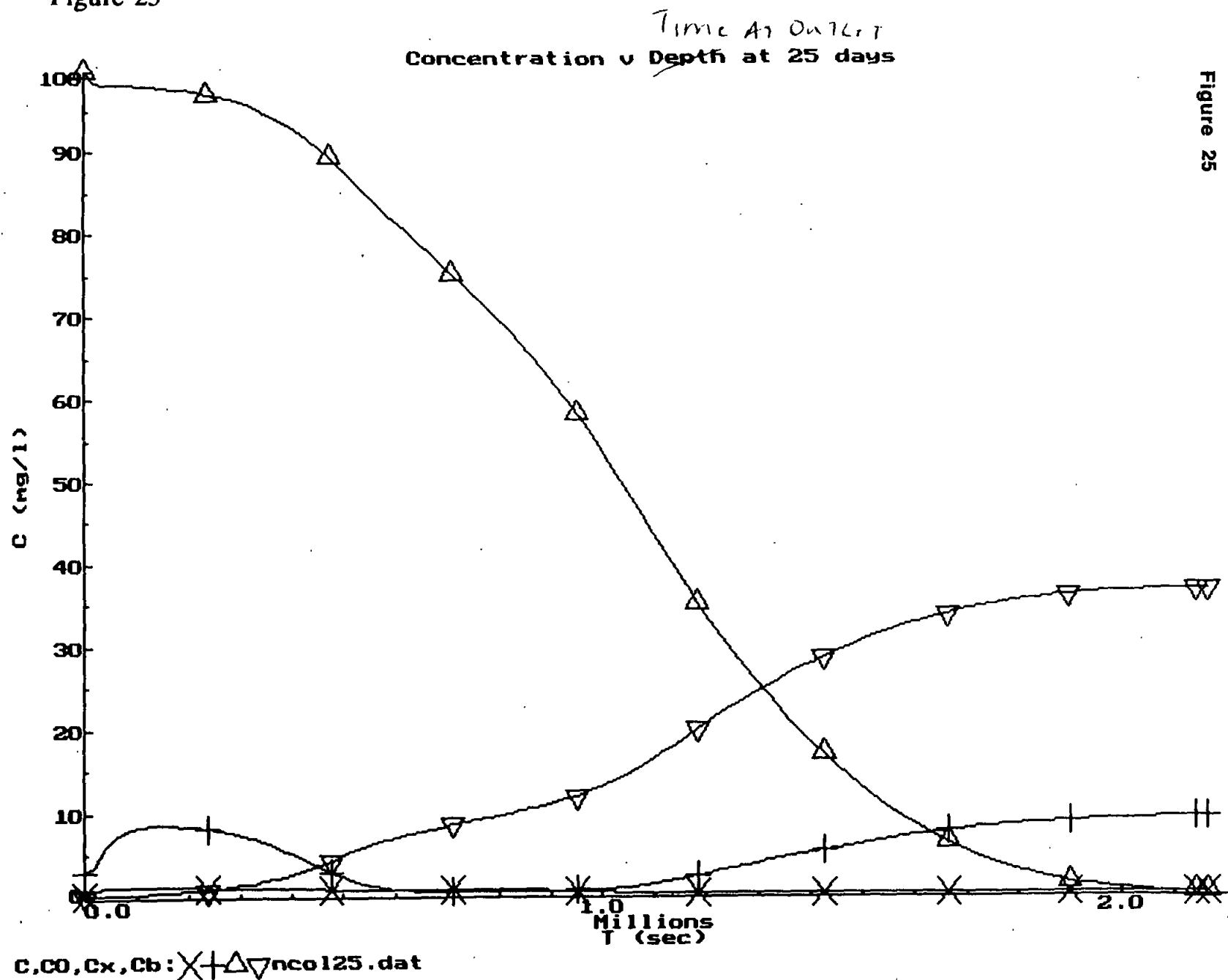


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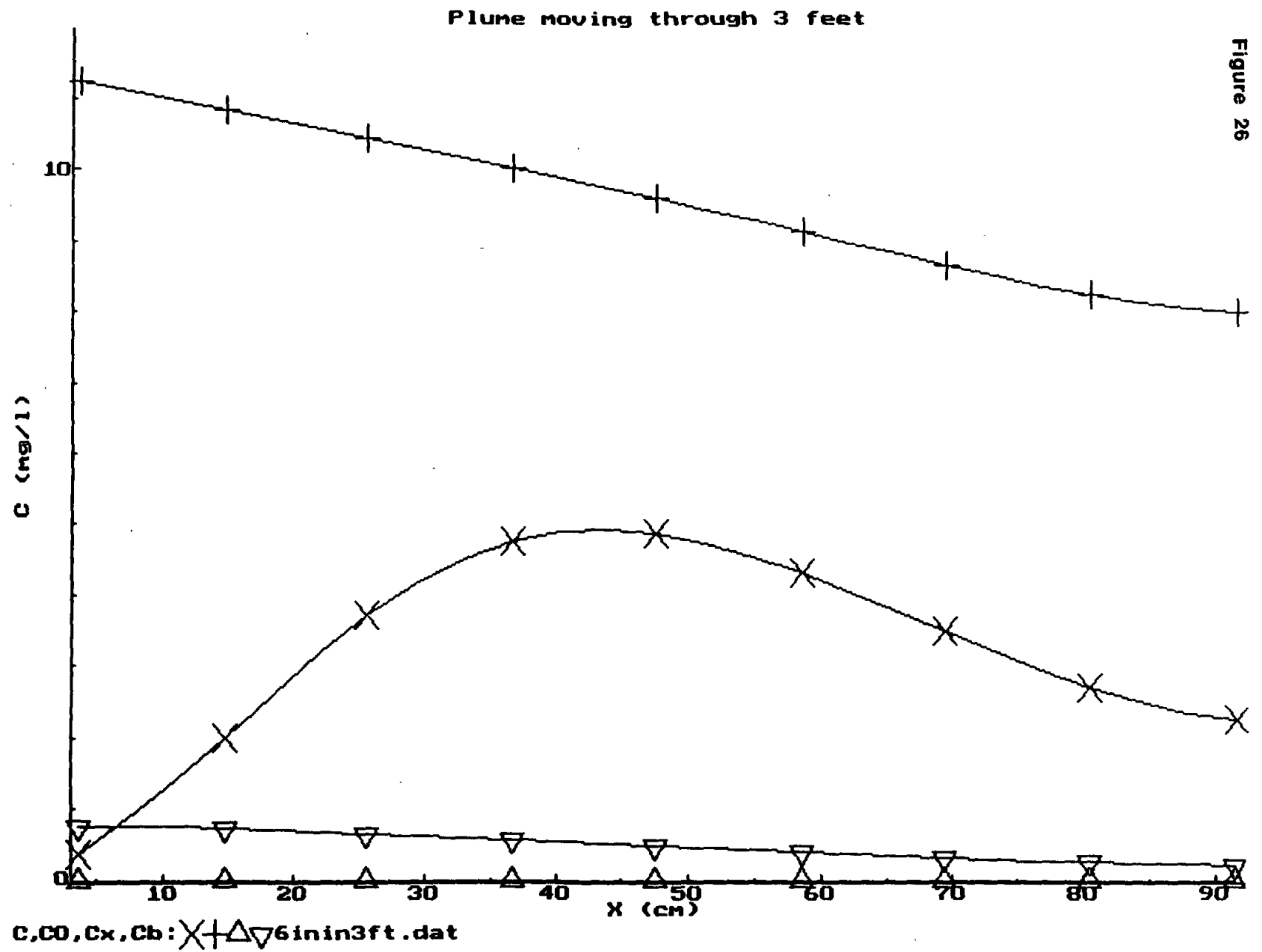


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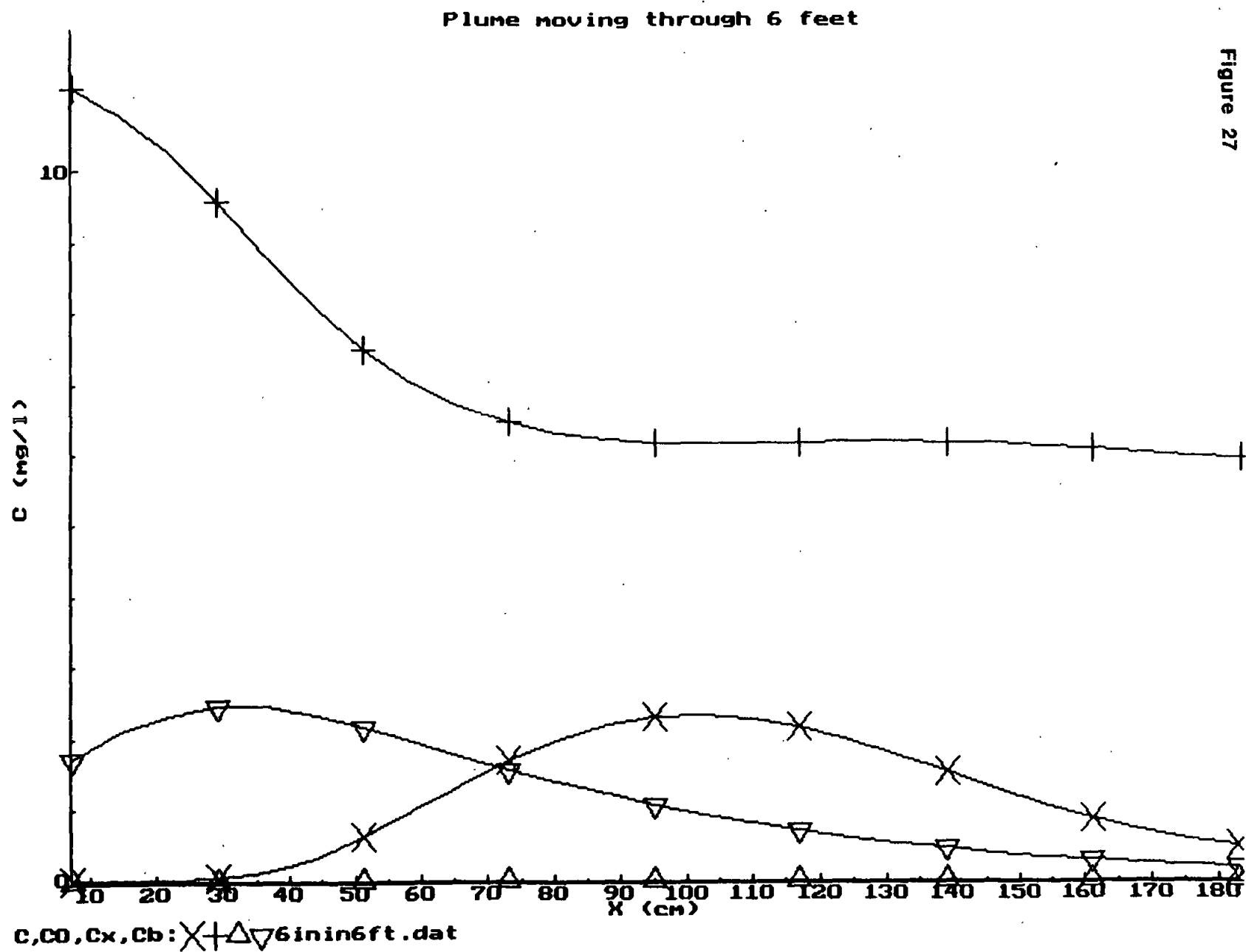


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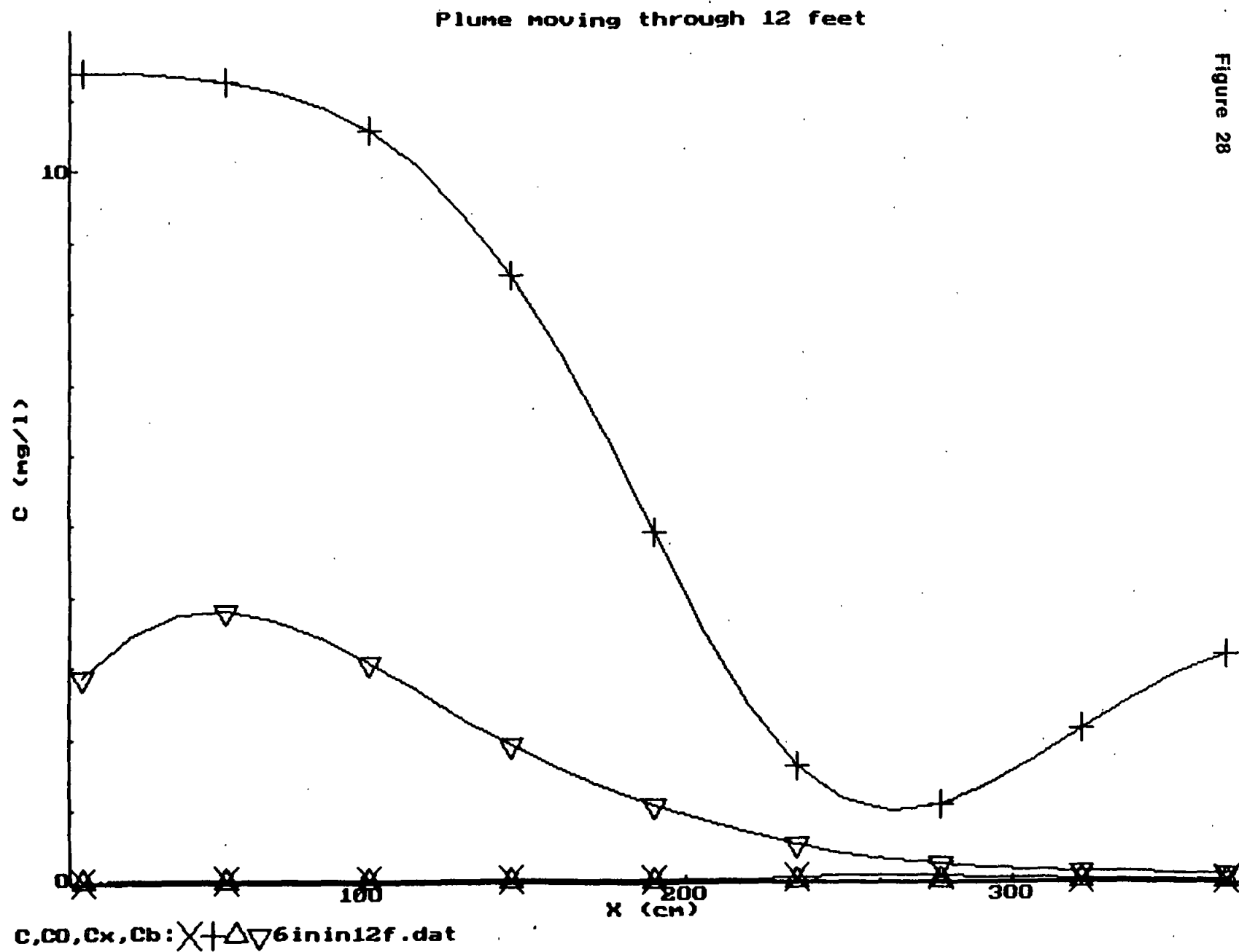


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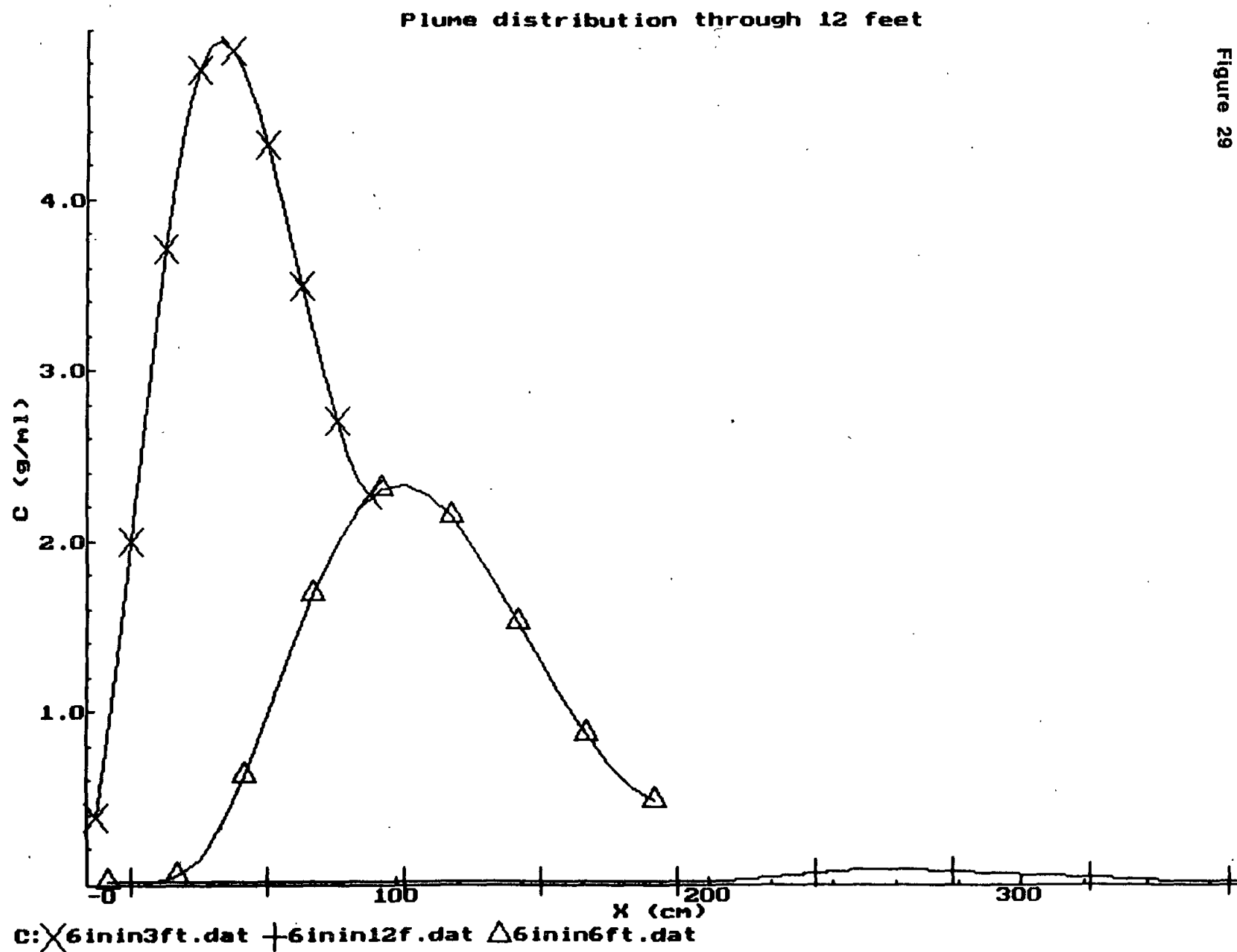


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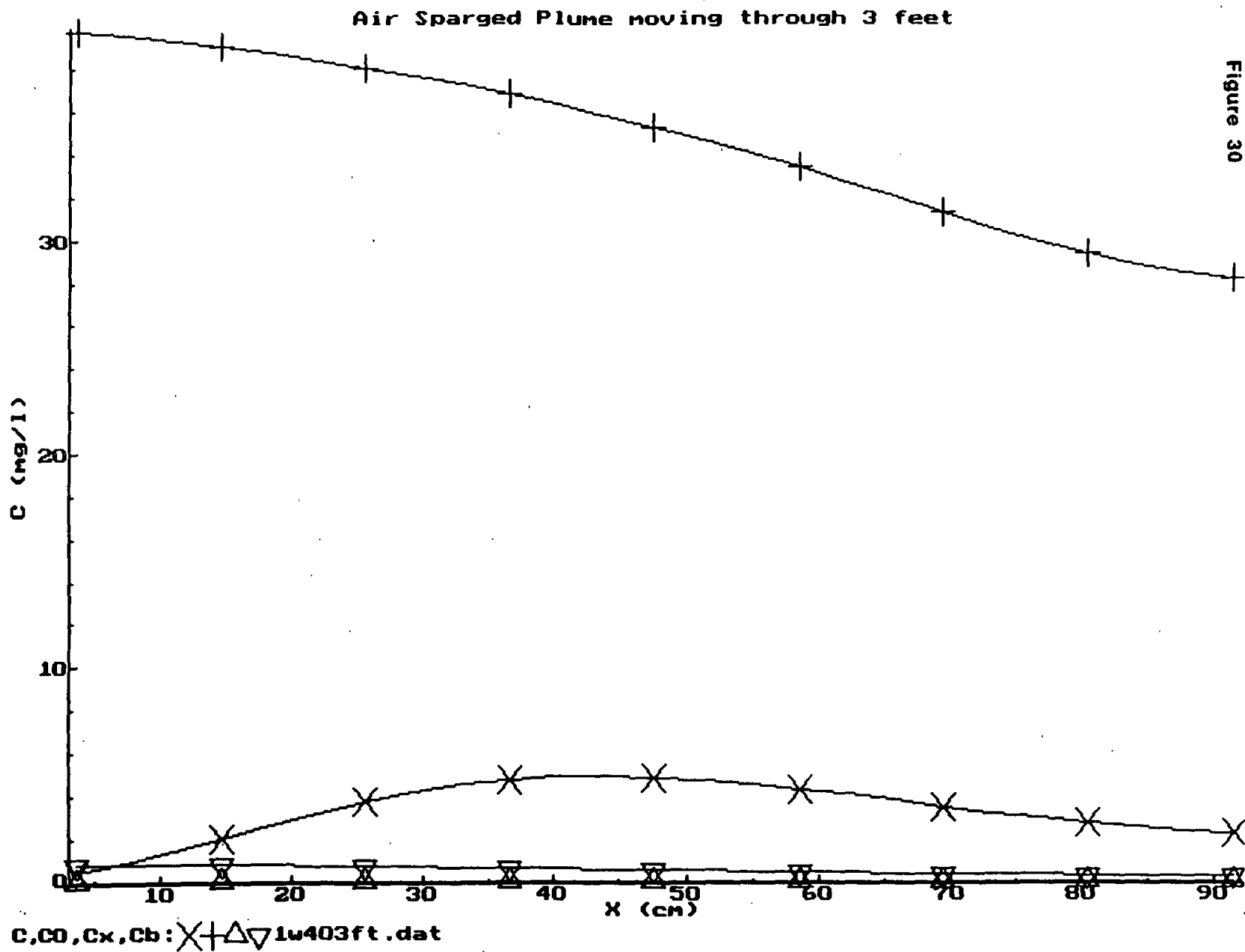


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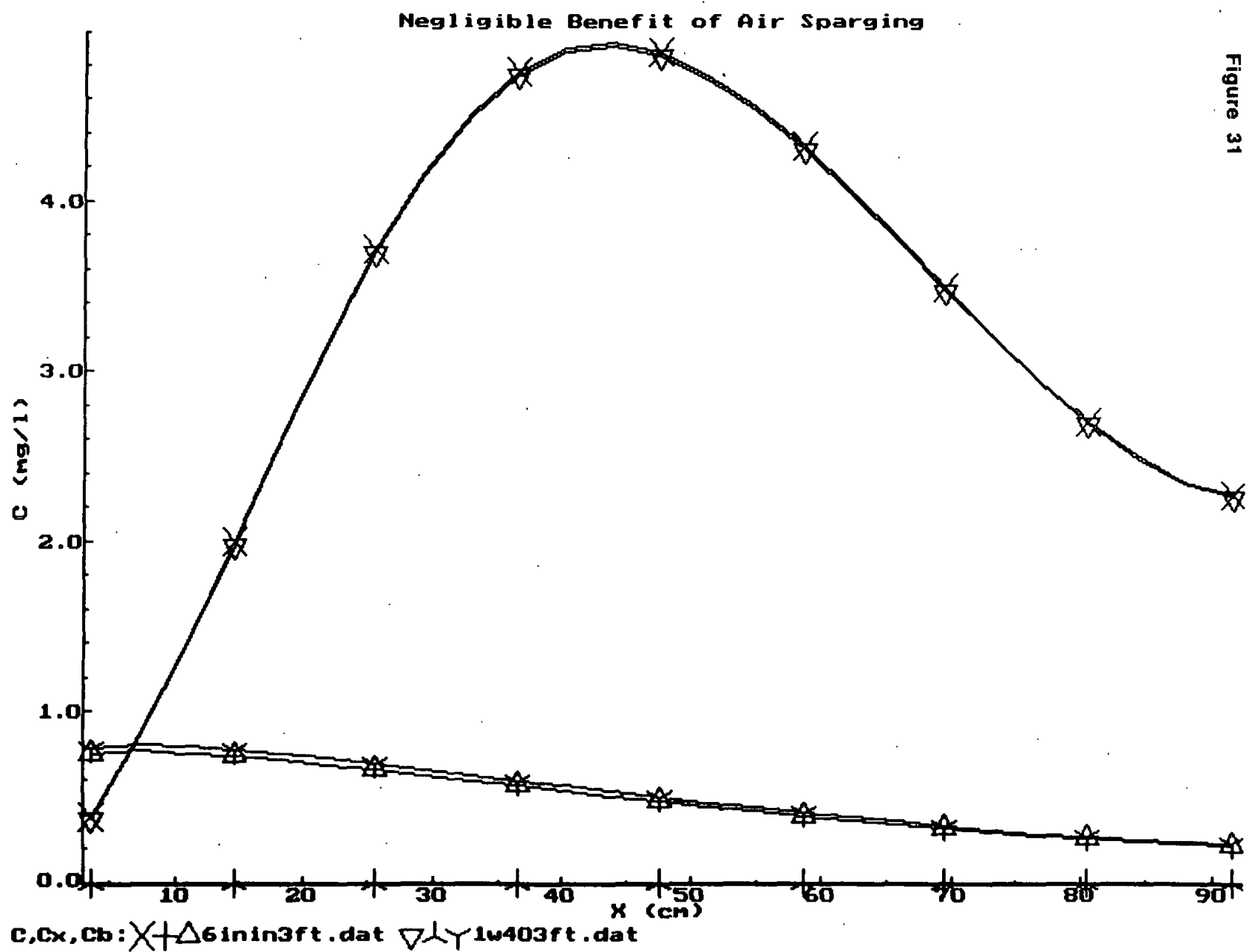


Figure 31

Figure 32

Air Sparged Plume moving through 6 feet

Figure 32

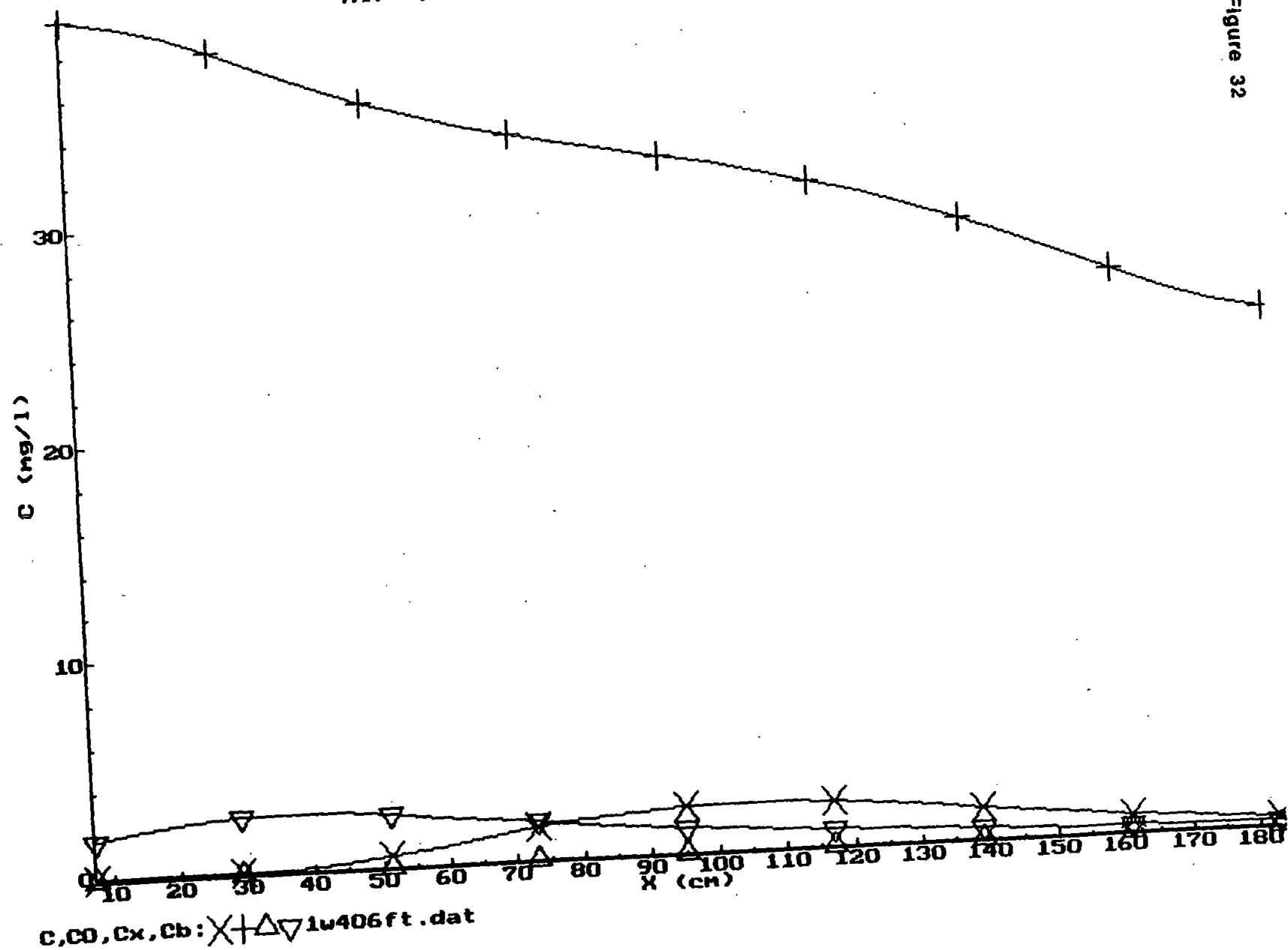


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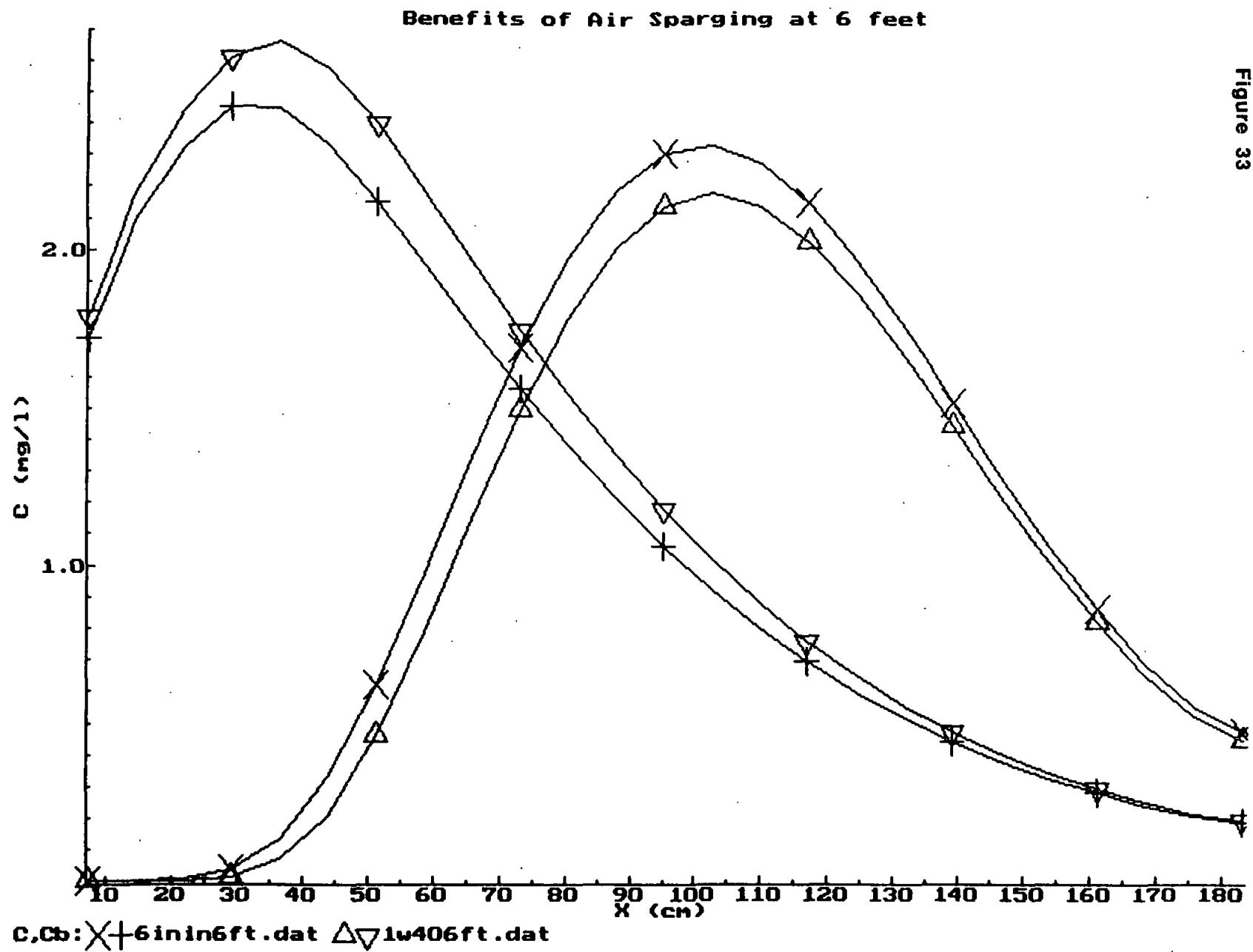


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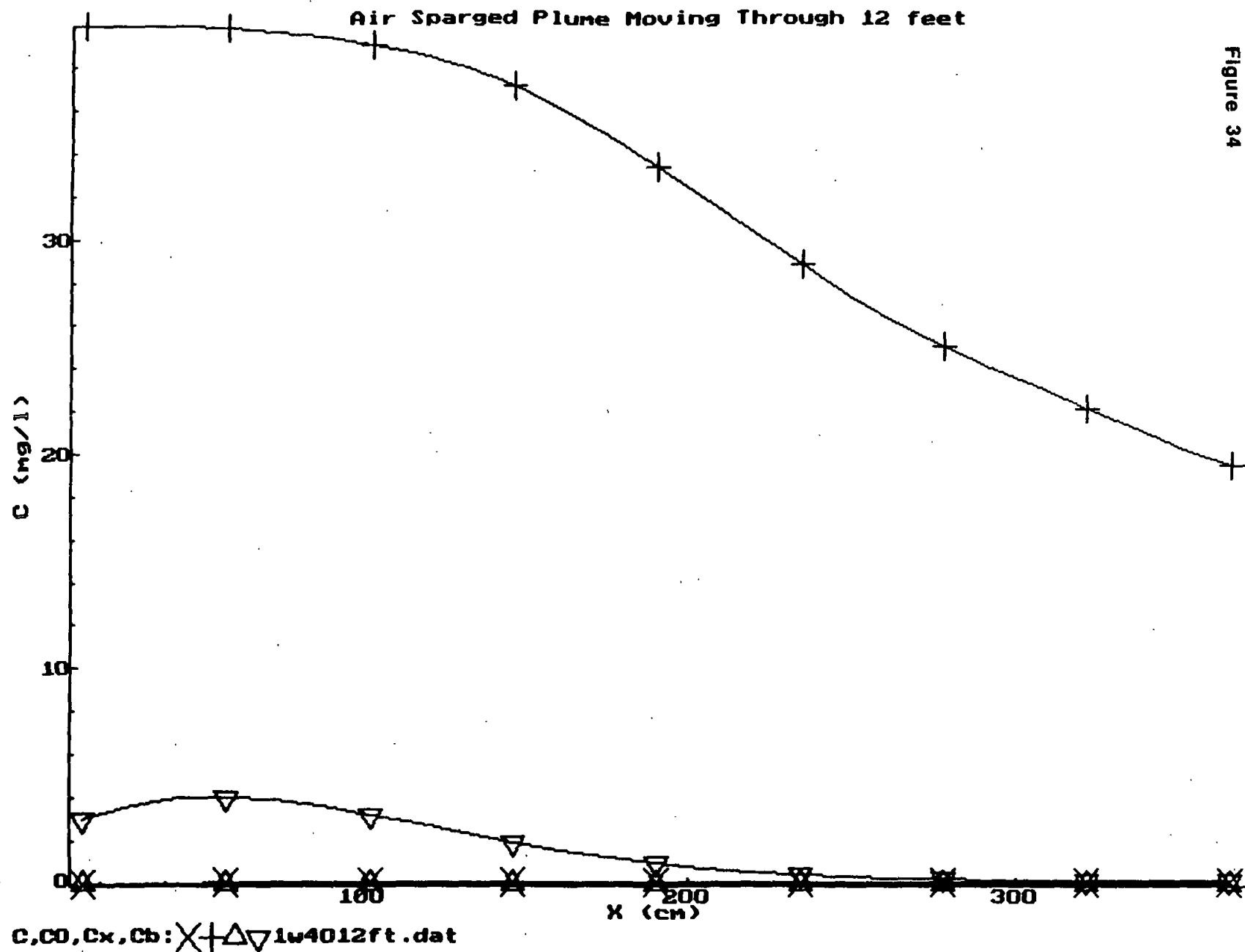


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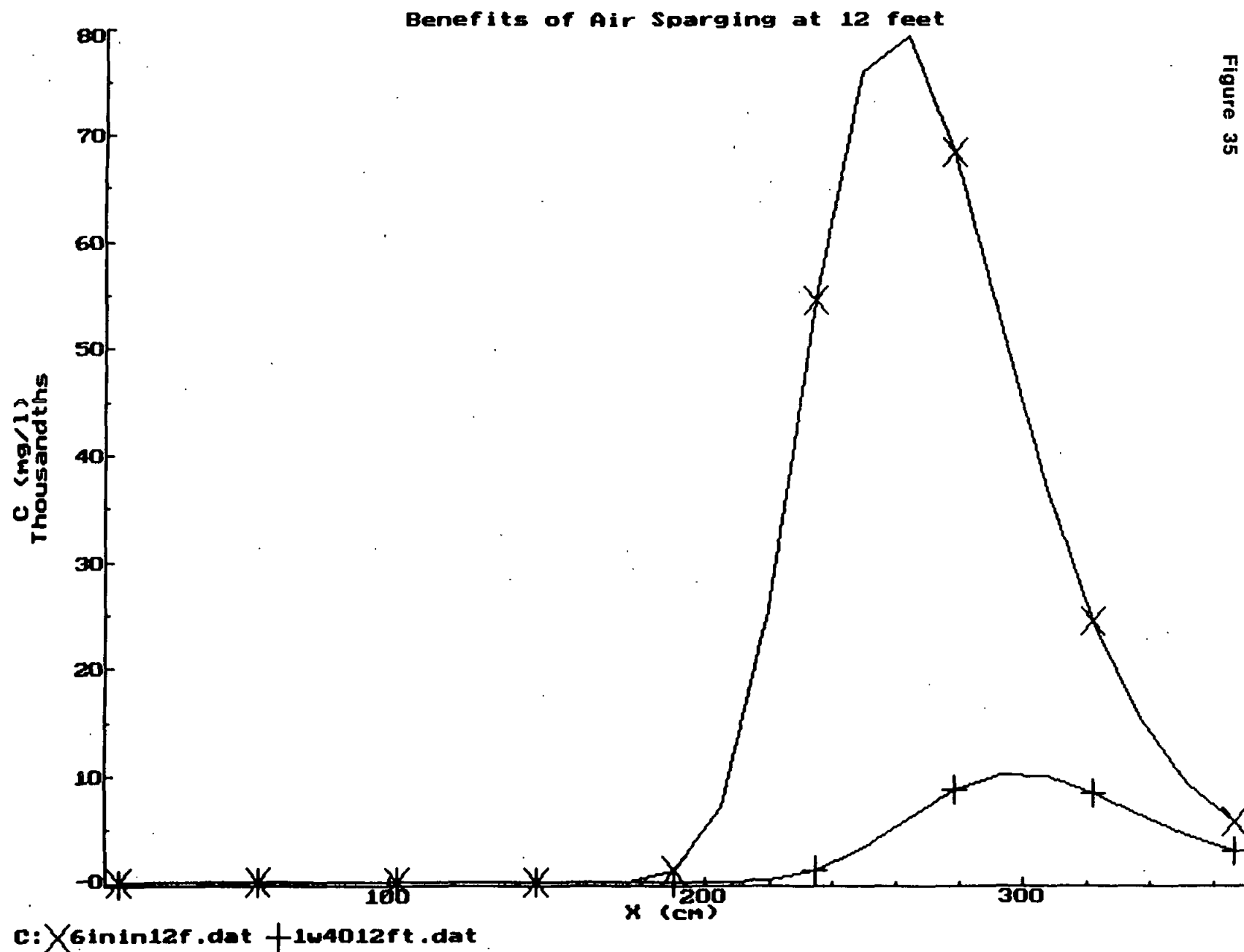


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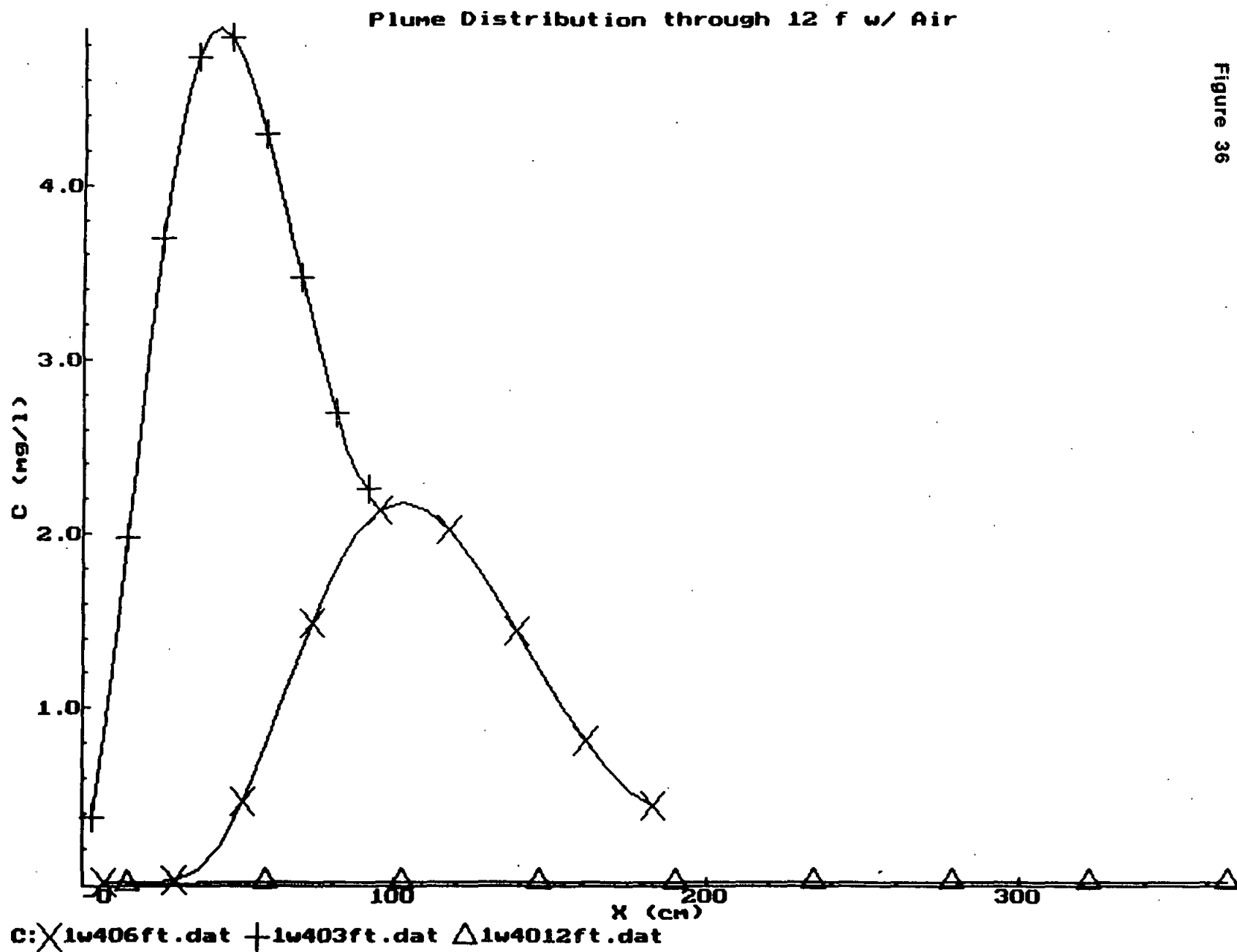


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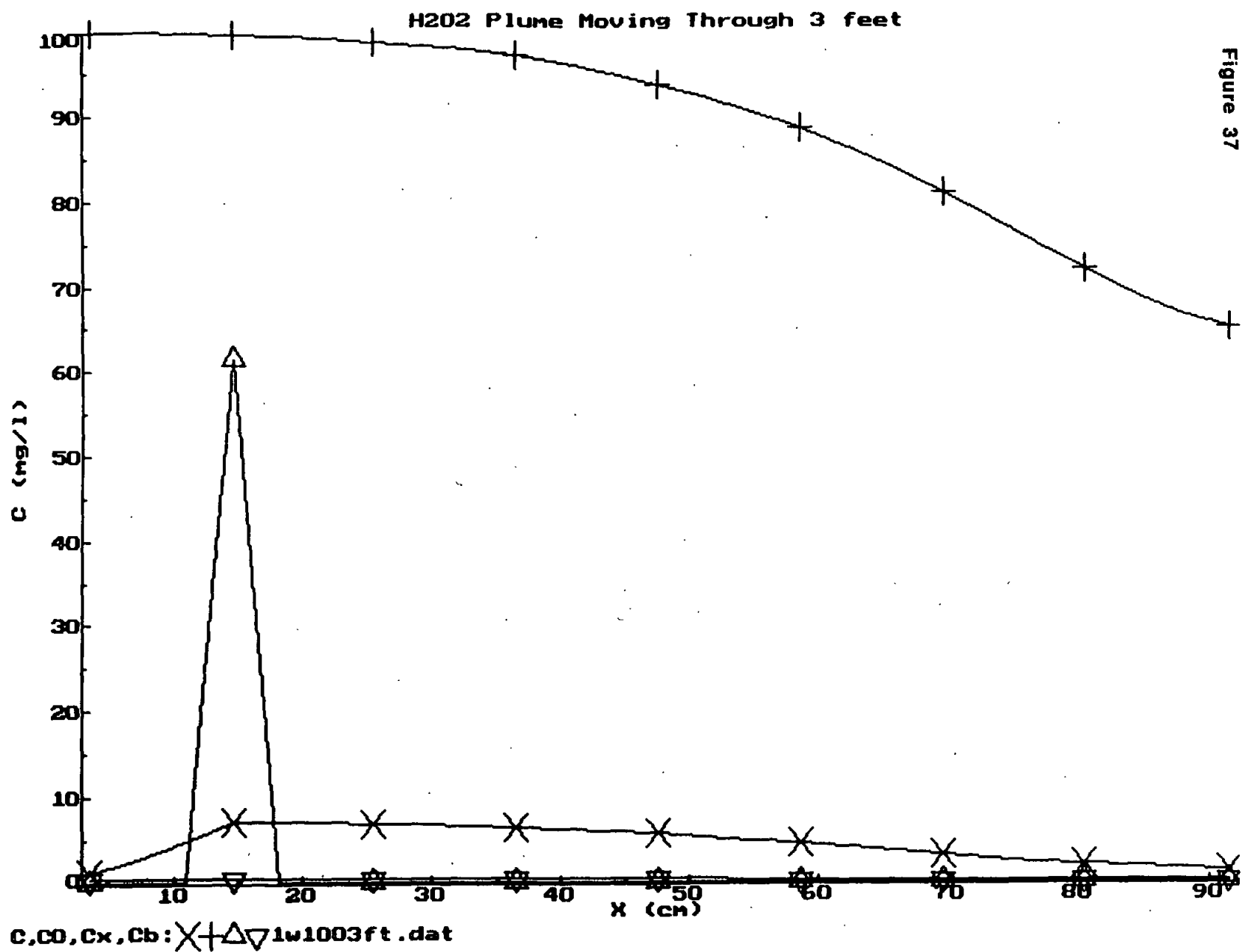


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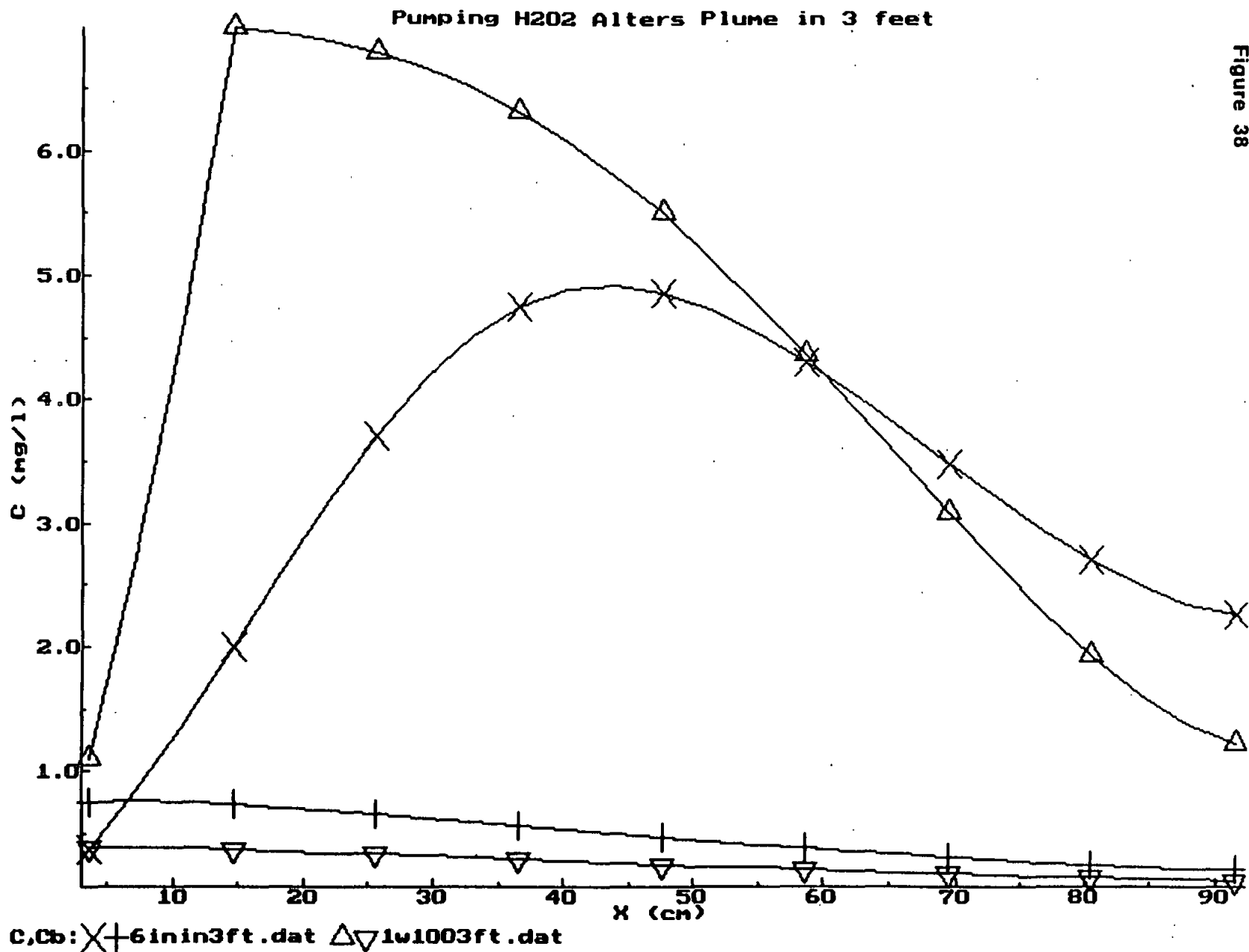


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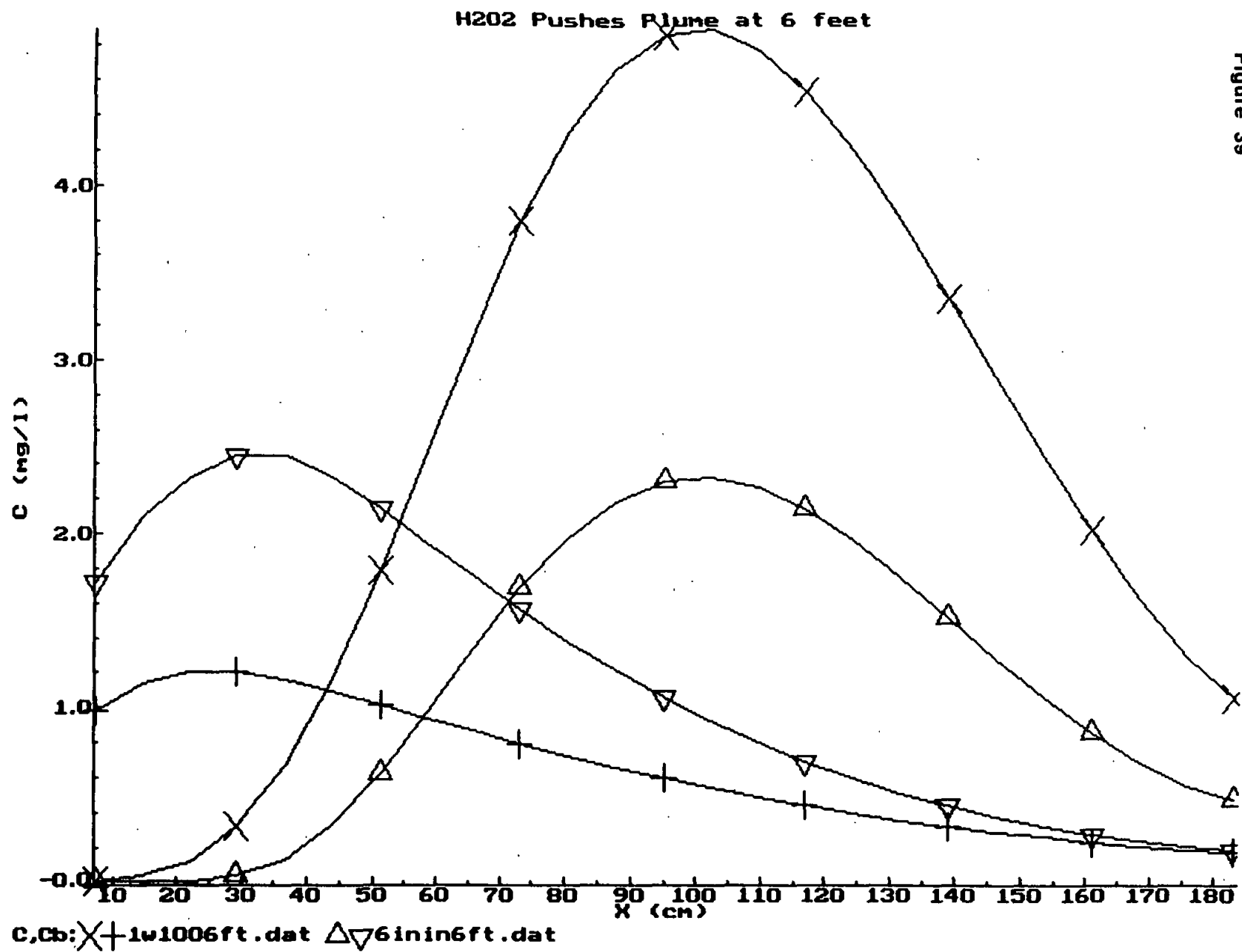
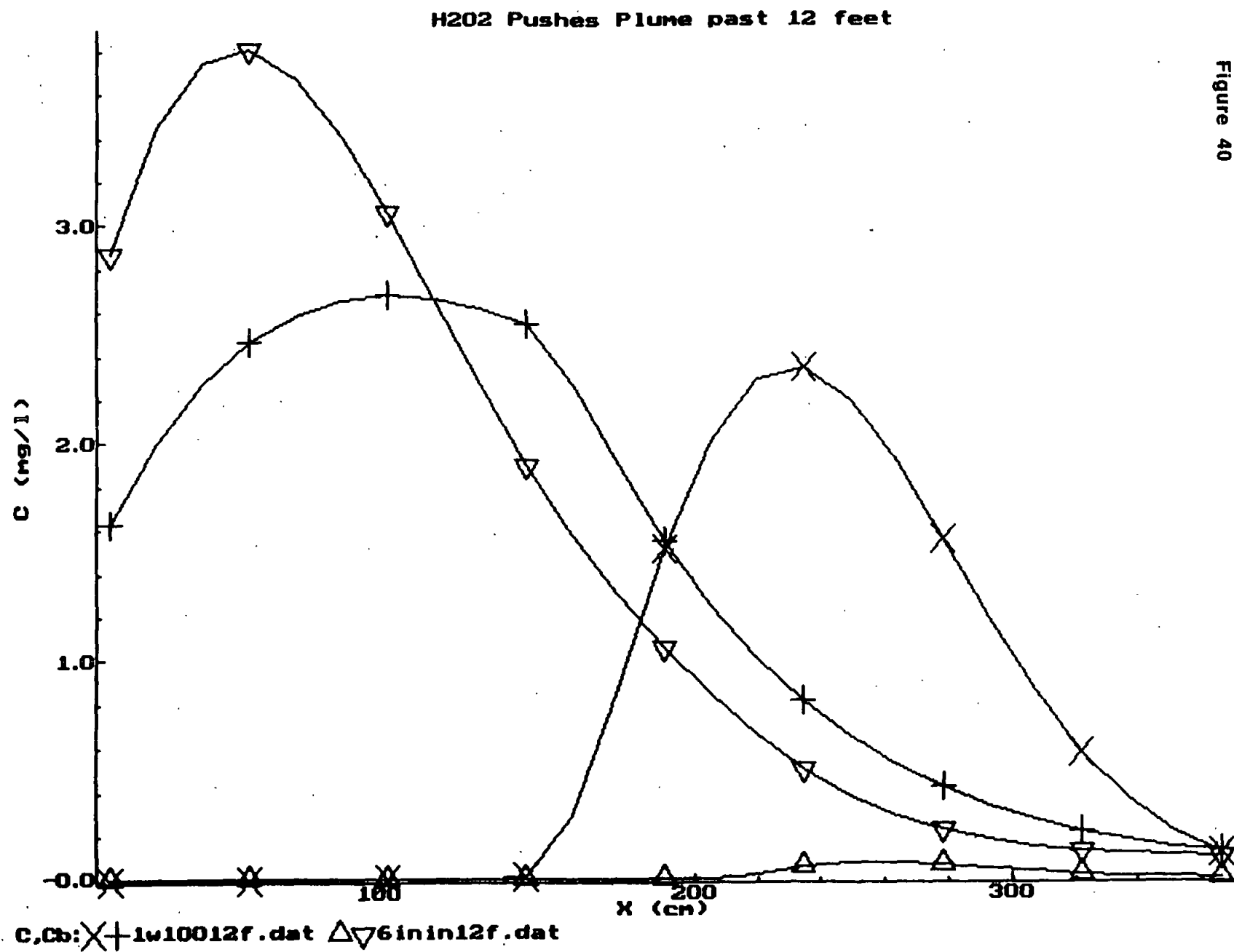


Figure 39

Figure 40



Section 6

Recommendations

As indicated in the introduction of this report, one of the goals of this study was to provide quantitative information about the Reilly Site to help answer the question "Is it technically feasible and engineering-wise practical to clean up parts of the site by implementing insitu bioremediation technology?". And if so: "How should insitu bioremediation be implemented on the site?"

Answers to these questions are discussed below. The first part summarizes and discusses the findings from the laboratory studies and the implications as regards feasibility and practicability. The second part outlines recommendations for a field demonstration of insitu bioremediation on the mounded parts of the site that was the focus of the laboratory study.

ASSESSMENT OF FEASIBILITY- PRACTICABILITY

As regards the feasibility question:

1. Laboratory data on a series of representative contaminated soil samples have demonstrated that polynuclear aromatic hydrocarbons (PAH's), which are the chemicals of concern on the Reilly Site, are biodegradable in soil and groundwater environments. The study shows that PAH's in percolating groundwater can be removed to below detection limits by the actions of ambient microorganisms.
2. Although the metabolic pathways of PAH biodegradation were not elucidated, direct evidence of insitu biodegradation-mineralization was demonstrated by spiking contaminated soils and groundwater with ¹⁴C-labeled PAH's and measuring ¹⁴C carbon dioxide production.
3. Analysis of spatial distributions of organics in the soil samples shows that the ratios of PAH's are very similar throughout the mounded site. This is not unexpected because historical records show that the site was primarily contaminated with

creosote type chemicals from wood treating operations . It can therefore be inferred that the laboratory test results are applicable for the whole site.

As regards the question of practicality:

Results from the laboratory studies have given quantitative insight on the effects of major environmental process variables on the rates of biodegradation. This information provides the basis for selection of engineering design criteria for field applications .

On the one hand the laboratory studies have provided explanations as to why the natural microbiological purification processes, as they currently exist on the site, have been and still are inadequate for cleaning up the site in a reasonable time frame. Specifically the results help explain why, even after half a century, high concentrations of PAH's persist throughout the site including the near surface soils. On the other hand, analysis of data from batch and continuous flow reactor test have identified and quantified the effects of controllable environmental factors that need to be modified to accelerate insitu bioremediation. Two types of limitations that are responsible for slowing down rates of biodegradation have been identified and described, namely,

1. limitations caused by insufficient localized concentrations of molecular oxygen;
2. operational limitations that stem from nonuniform flow distribution of water, partial or incomplete wetting of the contaminated soils, spatial variations in active biomass, and inadequate contact time.

Test data on minimally disturbed soil samples in continuous flow columns have helped identify critical operational limitations related to physical features of the soil. By contrast, continuous flow column microcosm studies along with batch reactor studies using well characterized mixed soils have provided critical information about the need for enrichment with oxygen to accelerate solubilization and bioremediation.

Mass balance studies on carbon removal, oxygen utilization, and carbon dioxide production have been used to calculate and correlate the time dependent disappearance of total and PAH related organic carbon; the former includes a variety of phenolics and heterocyclic organic chemicals typically present in creosote. This information, together with measurements of PAH's concentrations in the soil before and after column studies

where the soils had been subjected to percolation at groundwater flow conditions, has been used to project the time required to achieve a desired degree of cleanup of the soil. A related accomplishment of this study is that the data have been correlated using mathematical models. These models provide a rational basis for engineering design studies and for setting design criteria.

The overall conclusion of this study of Reilly Site soils is that insitu bioremediation is feasible and practical for removal of PAH's. What remains unclear is how reliably the laboratory test results can be scaled up to larger field scale areas such as the mounded site. Field applications will differ from the laboratory studies in several respects, namely,

1. Depth of the contaminated soil zone is approximately 30 feet compared with the two foot column studies used in the laboratory; the greater depth poses more difficult problems to ensure adequate supply of oxygen.
2. The mounded site is primarily unsaturated, being above the water table, whereas the laboratory studies were mostly run under saturation flow conditions. The presence of soil air in the mounded area is expected to be a major advantage because soil air is an additional source of molecular oxygen.
3. Ambient soil temperatures in the field are expected to be significantly lower than the laboratory conditions except for one of the studies which was carried out at 10-15°C. The latter studies show that there is an initial slow down of rates of biodegradation at lower temperatures but there also is evidence of compensation due to greater localized accumulations of biomass.

In view of these differences and considering that this site is in a high population area it is deemed prudent to initiate cleanup of the site with a scaled down field test, as outlined below, rather than a full scale clean up of the whole mounded site.

RECOMMENDATIONS FOR REILLY SITE FIELD TEST

Because the study focused on contaminated soils from the mounded area, it is recommended that a field demonstration of insitu biodegradation be carried out on this part of the site. The field test should be designed:

1. to implement insitu biodegradation in a limited size test plot under controlled conditions.
2. to control the flows of air and water into the test site to ensure that conditions are conducive to rapid solubilization and biodegradation of the pollutants without causing migration of pollutants into the saturated groundwater flowing beneath the site.
3. to instrument the test site for sampling ground water and air as well as taking occasional soil samples for analysis.
4. test site should be sized to be sufficiently large to provide a realistic field demonstration but small enough to allow extensive sampling and testing.
5. test site should be physically isolated from the rest of the site to prevent lateral migration of water and chemicals and offsite migration of pollutants. Installation of a partial grout curtain should be considered.
6. design of the site should include preliminary cost studies to help focus on low cost alternatives for oxygen addition and water injection.

SCHEMATIC OF FIELD DEMONSTRATION

A schematic visualization of the test site is shown in Figure 6.1.

The test plot should be located on the mounded site in the vicinity of the area where the drillings were carried out to obtain soils for the laboratory studies (see map in section I Appendix.)

Dimension of the test plot should be 15-20 feet in diameter and down to the water table which is approximately 30 feet.

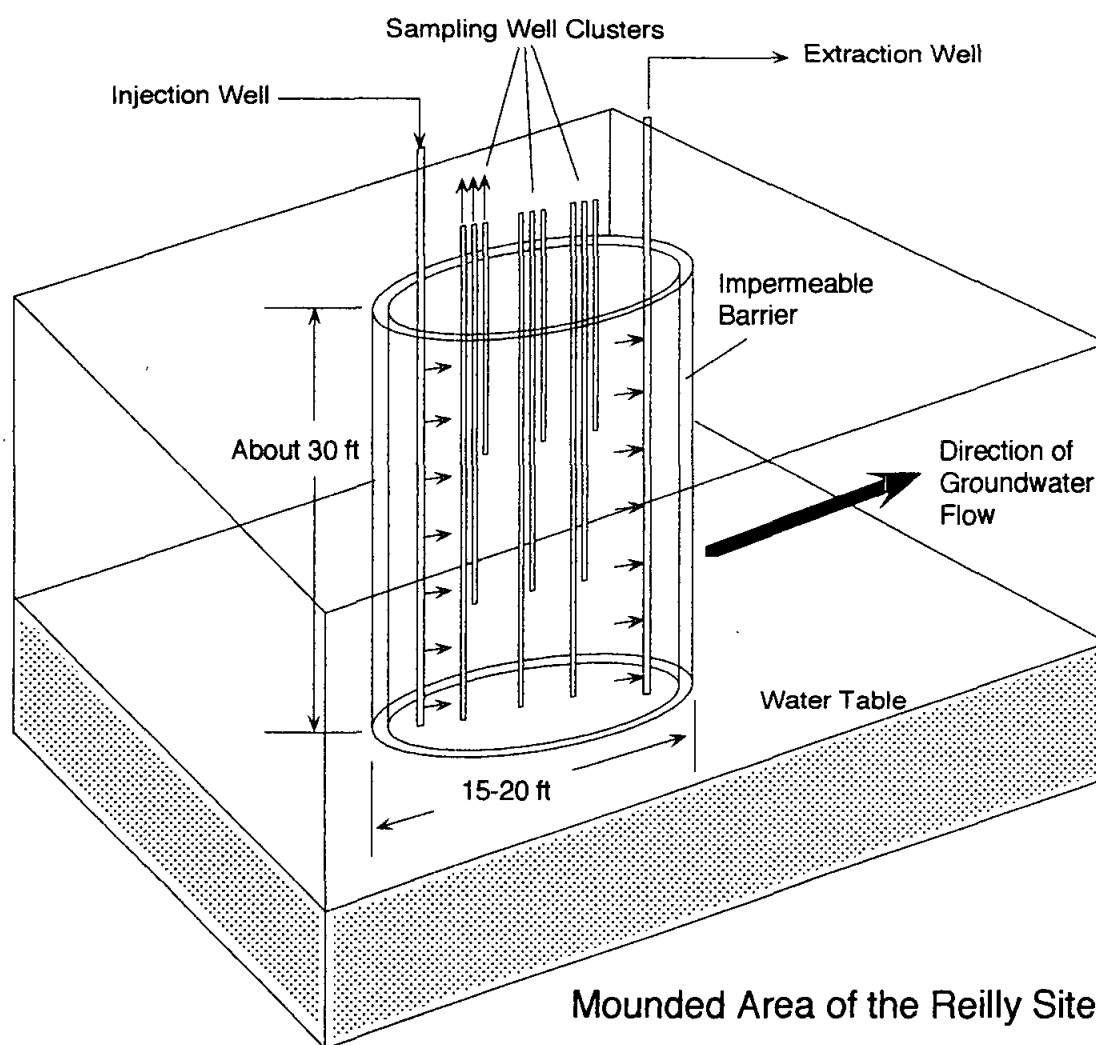
Pumping wells should be installed to provide water for irrigation of the soil column and to monitor the downgradient water quality.

A series of sampling wells to allow taking vertical profile samples of soil air and water from the unsaturated zone should be installed. These samples will be analyzed for carbon dioxide and oxygen as well as residual organics to monitor progress.

A major advantage of the proposed test plot is that it can also be used to evaluate the advantages of using some innovative ideas for accelerating insitu biodegradation that have been observed to be beneficial in the laboratory column studies.

Section 6 Figures

Figure 6.1 A Schematic Visualization of the Test Site for Field Demonstration of Bioremediation at the Reilly Mounded Area



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January 14, 1994

Mr. Douglas Beckwith
Project Manager
MPCA
520 Lafayette Road
St Paul, MN 55155-4194

Dear Mr. Beckwith

This letter is to formalize delivery of two draft copies of the final report describing the results of the Reilly Tar Site study carried out by the University of Minnesota.

The report presents the results of a two year study of creosote contaminated soils from the mounded area on the southwestern section of the site. The overall objective was to assess the feasibility and practicability of using insitu treatment based on microbial oxidation to biodegrade polynuclear aromatic hydrocarbons (PAH's) which are the chemicals of concern on this site.

The report includes descriptions of field sampling to obtain representative soil samples, descriptions of the protocols for measuring the physical-chemical characteristics of the soils, test data on numerous soil samples, as well as the results from bench scale laboratory testing of mixed and minimally disturbed soils. It also describes a new computer program (TPMBX2) for analyzing/interpreting/projecting the laboratory test data.

The last part of the report outlines recommendations for field implementation of the techniques developed in the course of the study for decontaminating the mounded area. Let me know what MPCA plans to do and call me if you want to discuss the implementation as suggested in the recommendations.

An important new feature of this study was the development of protocols for obtaining and testing minimally disturbed soil cores using stainless steel inserts in a split spoon sampler. The methods for sampling and laboratory testing have been documented in some detail because they may be useful at other sites.

As shown in the report, some of the split spoon insert soil columns have been tested under continuous groundwater flow conditions for well over a year. This long term testing has generated some very interesting information about the treatment response of variably contaminated soils over long periods of time. These extended time column studies have also allowed us to test and demonstrate the feasibility of ensuring complete biodegradation of solubilized PAH's by providing adequate supplies of oxygen. This is an important concept because it provides the basis for engineered field applications for insitu bioremediation while preventing offsite migration of these chemicals of concern thereby protecting regional groundwaters.

We had originally planned to also carry out process studies using a box type microcosm. However, this phase of the work plan was deferred because it was deemed more important to extend the column studies as long as possible. For your information, a boxtype microcosm has been constructed and its physical features are described in an addendum to this report. We would be interested in testing soils in this microcosm but additional sources of funding would have to be found. As indicated in the report, availability of dissolved oxygen in groundwater is probably the most critical limiting factor as regards implementation of insitu biodegradation. The boxtype microcosm is ideally suited for studies aimed at developing/refining strategies for oxygen addition in subsurface environments.

As you know, the total funding provided by MPCA for this project was \$70,000. Actual expenditures were considerably higher, furthermore the project benefitted from the University's waiving of indirect costs. I obviously underestimated the costs in the original proposal and another contributing factor was that the scope of work with minimally disturbed soil cores was expanded.

I have no regrets about the way this project has gone. It has generated a great deal of information and insight on the implementation of insitu biodegradation of the mounded area of the Reilly Site. The project has also benefitted a number of our graduate students. Their names are listed as coauthors in the report. Five of them are now professionally employed in the field of environmental cleanup.

Please review the report at your convenience.

Let me know if you want a copy of the TPMBX2 program. The program user friendly and may be of interest to MPCA staff in your section.

It has been a pleasure working with you and your associates.

Sincerely,

Walter J. Maier

Walter J. Maier
Professor Civil Engineering